Ovarian cancer. Biomarkers, surgical outcome and survival.

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Biomarkers, surgical outcome and survival

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Ovarian cancer

Ovarian cancer is the most lethal of the gynaecological malignancies. Improved surgical and oncological treatment has prolonged life after diagnosis, but long-term survival remains poor. This thesis explores new biomarkers for improved ovarian cancer diagnostics, evaluates surgical outcomes after the centralisation of ovarian cancer surgery in Sweden 2012 and analyses the incidence and survival trends in epithelial ovarian cancer in Sweden 1960 to 2014.

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Biomarkers, surgical outcome and survival

Pia Leandersson

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UNIVERSITY

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Abstract

Ovarian cancer is the most lethal of the gynaecologic malignancies. Around 700 women are diagnosed in Sweden per year. Most of the patients are diagnosed with late-stage epithelial ovarian cancer (EOC) and the prognosis is poor, with a five-year survival of 49%. Biomarkers for screening and early diagnosis have been sought for decades. The biomarkers in clinical practice today, CA125 and HE4, lack sensitivity and specificity for early-stage EOC. The standard treatment for EOC is primary surgery with adjuvant chemotherapy. Centralisation to high-volume hospitals with subspecialist surgeons and improved chemotherapy regimens have improved outcome and survival. Ovarian cancer surgery was centralised in Sweden in 2012.

The aims of this thesis were to assess new biomarkers for their potential to improve the diagnostic performance of CA125 and HE4 in women with ovarian tumours (studies I and II), to evaluate ovarian cancer surgery after centralisation (study III) and to analyse incidence and survival in EOC in Sweden since the 1960s (study IV).

Study I: CA125, HE4, B7-H4 and suPAR were analysed in preoperative plasma samples from 350 women with ovarian tumours. Plasma levels of CA125, HE4 and suPAR(II-III) increased from benign tumours to borderline, EOC type I and EOC type II. A logistic regression model combining CA125, HE4, suPAR(II-III) and age performed better than the established Risk of Ovarian Malignancy Algorithm (ROMA) for discrimination of benign tumours from EOC in premenopausal women. The ROMA performed best in postmenopausal women. High preoperative levels of HE4, CA125 and suPAR(I) were prognostic for poor survival after EOC diagnosis. In women above 75 years, high suPAR(I) indicated very poor prognosis in the first year after diagnosis. *Study II:* In this study, 177 inflammation- and cancer-associated biomarkers were analysed in preoperative plasma samples from 180 women with ovarian tumours, using the proximity extension assay. HE4 was the best performing single biomarker for discrimination between benign tumours and EOC. Three-biomarker combinations of HE4, CA125 and one additional biomarker were compared to a reference model of HE4 plus CA125. No biomarker significantly improved the diagnostic performance of HE4 plus CA125. *Study III:* Out of 1108 cases of ovarian surgery with curative intent reported to the GynOp Registry in 2013-15, 30% were performed in regional hospitals with fewer than 20 cases per year. Compared with regional hospitals, tertiary centres perform more extensive surgery without an increased frequency of major complications. Large differences exist in patient selection for primary surgery and complete resection rates between the tertiary centres. *Study IV:* All women with a diagnosis of epithelial ovarian, fallopian tube, and peritoneal cancers or undesignated abdominal/pelvic cancer from 1960 to 2014 in the Swedish Cancer Registry were identified. Analyses of age-standardised incidence and relative survival (RS) were carried out and time trend graphs modelled according to age, tumour site, and morphology. Since 1980, the age-standardised incidence of EOC has declined in Sweden. Survival in EOC up to five years from diagnosis improved from 1960 to 2014. The 10-year survival has remained unchanged since 1960.

Key words epithelial ovarian cancer, biomarkers, ovarian cancer surgery, incidence, survival

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Thesis at a glance

Paper	Aim	Results	Conclusions
Paper I: A biomarker panel increases the diagnostic performance for epithelial ovarian cancer types I and II in young women.	To assess preoperative plasma levels of a panel of four biomarkers (CA125, HE4, B7-H4, suPAR) in relation to EOC types I and II and, secondly, to evaluate the biomarkers' ability to discriminate between benign and malignant tumours and to predict prognosis in EOC.	Plasma levels of CA125, HE4 and suPAR(II-III) increased from benign tumours to borderline, EOC type I and EOC type II. B7-H4 was increased in EOC II compared with benign ovarian tumours. In premenopausal women a model combining suPAR(II-III), HE4, CA125 and age had the highest diagnostic performance with AUC=0.933 for discrimination between benign tumours and borderline/EOC (p=0.007 for comparison with the ROMA). In postmenopausal women the ROMA performed best (AUC 0.914). High preoperative levels of HE4, CA125 and suPAR(I) were prognostic for poor survival after EOC diagnosis. High suPAR(I) was an independent marker for poor prognosis in the first year after diagnosis. Women above 75 years with high suPAR(I) had a very poor prognosis (HR=8.9, p=0.01).	The plasma levels of CA125, HE4 and suPAR(II-III) increase from benign tumours to borderline tumours, EOC type I and EOC type II. A biomarker panel with suPAR(II-III), HE4, CA125 and age discriminates benign from malignant ovarian tumours, including borderline, with higher diagnostic accuracy compared to the ROMA in premenopausal women. High suPAR(I) predicts poor short-term survival after EOC diagnosis, especially in elderly women.
Paper II: A multiplex biomarker assay does not improve the diagnostic performance of HE4 and CA125 in ovarian tumour patients.	To search for new candidate biomarkers with the potential to improve performance of CA125 and HE4 for discrimination between benign and malignant ovarian tumours.	177 biomarkers were analysed with the proximity extension assay. HE4 had the best diagnostic performance of the individual markers with AUC 0.771 (benign tumours vs. borderline/EOC) and AUC 0.837 (benign tumours/borderline vs. EOC). A reference model with HE4 and CA125 (AUC 0.773 and 0.837) was compared to a model of HE4, CA125 and one additional biomarker. No additional biomarker led to significant improvement ($p < 0.05$), for any comparison.	HE4 is the best performing single marker for discrimination between benign and malignant ovarian tumours. No biomarker significantly improved the combined diagnostic performance of HE4 plus CA125 in our study.
Paper III: Ovarian cancer surgery - a population-based registry study.	To evaluate ovarian cancer surgery in Swedish tertiary centres and regional hospitals 2013-15.	Four tertiary centres (TCs) and 21 regional hospitals (RHs) reported 1,108 cases of surgery with curative intent, 770 cases in TCs and 338 cases in RHs. In advanced EOC, non-gynaecological additional cytoreductive surgery was more common in TC, 44.9% vs. 21% of RH patients ($p<0.001$). Selection of patients for primary debulking surgery (PDS) (45% to 93%, $p<0.001$) and complete resection rates in PDS (36% to 70%, $p<0.001$) differed between the four TC. Major complications, re-admissions and re-operation rates did not differ between TC and RH.	In 2013-15, 30% of patients with ovarian malignancy had primary surgery at regional hospitals with less than 20 cases per year in the regions reporting to the GynOp Registry. TCs perform more extensive surgery without increased frequency of major complications compared with RHs. Large differences exist in patient selection for PDS and complete resection rates between centres.
Paper IV: Time trends for incidence and survival of epithelial ovarian, fallopian tube, peritoneal, and undesignated abdominal/pelvic cancers in Sweden 1960–2014.	To analyse time trends for incidence and survival of EOC in Sweden in 1960-2014 with regard to age, tumour site and morphological subgroup.	The overall incidence of ovarian, tubal, peritoneal, and undesignated abdominal/pelvic cancers declined from 1980. The median age at diagnosis increased. Serous carcinoma increased in incidence. RS at 1, 2 and 5 years from diagnosis improved from 1960, although not for the youngest and the oldest patients. Ten-year RS did not improve.	Since 1980 the incidence of EOC has declined in Sweden. Better surgical and oncological treatment has improved survival in EOC up to five years from diagnosis since 1960. However, long-term survival remains poor.

List of original papers

This thesis is based on the following papers, referred to by their Roman numerals.

I. Leandersson P, Kalapotharakos G, Henic E, Borgfeldt H, Petzold M, Høyer-Hansen G, Borgfeldt C. A biomarker panel increases the diagnostic performance for epithelial ovarian cancer types I and II in young women. *Anticancer Research*. 2016;36(3):957-65.

II. Leandersson P, Åkesson A, Hedenfalk I, Malander S, Borgfeldt C. A multiplex biomarker assay does not improve the diagnostic performance of HE4 and CA125 in ovarian tumour patients. *Submitted.*

III. Leandersson P, Granåsen G, Borgfeldt C. Ovarian cancer surgery - A population-based registry study. *Anticancer Research*. 2017;37(4):1837-45.

IV. Leandersson P, Högberg T, Dickman P, Malander S, Borgfeldt C. Time trends for incidence and survival of epithelial ovarian, fallopian tube, peritoneal, and undesignated abdominal/pelvic cancers in Sweden 1960–2014. *Submitted*.

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The following publication is not included in the thesis but is of relevance to the field:

Noer MC, Leandersson P, Paulsen T, Rosthøj S, Leisby Antonsen S, Borgfeldt C, Høgdall C. Confounders Other Than Comorbidity Explain Survival Differences in Danish and Swedish Ovarian Cancer Patients - A Comparative Cohort Study. *Acta Oncol.* 2018 Aug;57(8):1100-1108.

Abstract

Ovarian cancer is the eighth most common female cancer worldwide and the most lethal of the gynaecologic malignancies. Around 700 women are diagnosed in Sweden per year. Due to vague symptoms most of the patients are diagnosed with late-stage epithelial ovarian cancer (EOC) and prognosis is poor, with a five-year survival of 49%. However, for early-stage EOC the prognosis is excellent. Biomarkers for screening and early diagnosis have been sought for decades. To date, CA125 and HE4 are the only biomarkers in clinical use. Both lack sensitivity and specificity for early-stage EOC. The standard treatment for EOC is primary surgery with adjuvant chemotherapy. Centralisation of ovarian cancer care to high-volume hospitals with subspecialist surgeons and improved chemotherapy regimens have improved outcome and survival. Ovarian cancer surgery was centralised in Sweden in 2012.

The aims of my thesis were to assess new biomarkers for their potential to improve the diagnostic performance of CA125 and HE4 in women with ovarian tumours (studies I and II), to evaluate ovarian cancer surgery after centralisation (study III) and to analyse the incidence and survival in EOC in Sweden since the 1960s (study IV).

Study I: CA125, HE4, B7-H4 and cleaved and intact suPAR were analysed in preoperative plasma samples from 350 women with ovarian tumours. Plasma levels of CA125, HE4 and suPAR(II-III) were found to increase from benign tumours to borderline, EOC type I and EOC type II while B7-H4 was only elevated in EOC II. Logistic regression models were fitted and a model combining CA125, HE4, suPAR(II-III) and age performed better (AUC=0.933) than the established ROMA algorithm (CA125, HE4 and menopause status) for discrimination of benign tumours from EOC in premenopausal women. The ROMA performed best in postmenopausal women (AUC=0.914).

Furthermore, we correlated preoperative biomarker levels with survival after EOC diagnosis. High HE4, CA125 and suPAR(I) were prognostic for poor survival. At 12 months suPAR(I) was the only independent biomarker prognostic for poor short-term survival. In women above 75 years, high suPAR(I) indicated very poor prognosis in the first year after diagnosis (HR=8.9, p=0.01).

Study II: 177 inflammation- and cancer-associated biomarkers were analysed in preoperative plasma samples from 180 women with ovarian tumour, using the proximity extension assay. HE4 was the best performing single biomarker for discrimination between benign tumours and EOC. Three-biomarker combinations of HE4, CA125 and one additional biomarker were compared to a reference model of

HE4 and CA125. No biomarker significantly improved the combined diagnostic performance of HE4 and CA125.

Study III: We analysed data from the GynOp Registry 2013-15. Out of 1108 cases of ovarian cancer surgery with curative intent, 30% were performed in regional hospitals with fewer than 20 cases per year. Four tertiary centres performed more than 25 surgeries per year. Compared with regional hospitals, tertiary centres perform more extensive surgery without an increased frequency of major complications. Large differences exist in patient selection for primary surgery and complete resection rates between the tertiary centres.

Study IV: We identified all women with a diagnosis of epithelial ovarian, fallopian tube, and peritoneal cancers or undesignated abdominal/pelvic cancer from 1960 to 2014 in the Swedish Cancer Registry. Analyses of age-standardised incidence and relative survival (RS) were carried out and time trend graphs were modelled according to age, tumour site, and morphology. Since 1980 the age-standardised incidence of EOC has declined in Sweden. The age-standardised RS in EOC up to five years from diagnosis improved from 1960 to 2014. The 10-year RS has remained unchanged since 1960.

In conclusion, CA125 plus HE4 continues to stand out as the best biomarker combination for assessment of cancer risk in a woman with ovarian tumours. CA125, HE4 and suPAR(I) are potential prognostic markers. Adding biomarkers to the preoperative assessment, especially in elderly women, could aid in the treatment decision on extensive primary surgery or neoadjuvant treatment.

After centralisation of ovarian cancer surgery in Sweden, many women still have surgery at low-volume regional hospitals. The treatment for advanced EOC seems to differ considerably between the tertiary centres. Further centralisation as well as increased collaboration and exchange of knowledge between tertiary centres are needed to ensure equal access to care, regardless of region of living.

Improved surgical and oncological treatment has prolonged life after EOC diagnosis. However, long-term survival remains poor. Most patients will die of their cancer. In order to cure EOC we need to find the patients at early stages. Better diagnostic tools are urgently needed.

Sammanfattning på svenska

Äggstockscancer är den åttonde vanligaste cancer bland världens kvinnor och den dödligaste av de gynekologiska cancersjukdomarna. Närmare 700 kvinnor drabbas årligen i Sverige, de flesta efter menopaus. På grund av vaga och ospecifika symptom får de flesta sin diagnos först när sjukdomen har spridd sig utanför bäckenet, och prognosen är därför dålig med en 5-års överlevnad på 49%. 90% av äggstockscancer utgörs av så kallad epitelial äggstockscancer (EOC). Merparten av EOC uppstår i äggledarna, och epitelial cancer i äggstockar, äggledare och bukhinnan anses vara olika uttryck av samma sjukdom, EOC.

EOC innefattar flera olika tumörtyper som kan delas in i EOC typ I (25%) och typ II (75%). Typ I är relativt snälla tumörer, som växer långsamt och ofta har en god prognos. Typ II är snabbväxande tumörer som oftast är spridda vid diagnostidpunkten. EOC typ II står för 90% av dödsfallen i EOC.

Om vi kunde hitta cancern i tidigare skede, innan spridning, skulle dödligheten i äggstockscancer förbättras mycket. Proteiner som frisätts av cancerceller eller omgivande vävnad vid cancersjukdom, kan mätas i ett blodprov. Så kallade protein biomarkörer, som kan ge information om cancer redan innan symptom, och därmed användas för screening, eller ge information om risken för cancer vid en misstänkt äggstockstumör, har man letat efter i årtionden. Ingen fungerande screening för äggstockscancer finns än. I dag används två markörer, CA125 och HE4, i riskbedömningen av äggstockstumörer. Tyvärr har båda markörerna låg känslighet för tidiga stadier av EOC, där det skulle ge störst effekt att hitta patienterna.

Standardbehandling för EOC är primär kirurgi följt av cellgiftbehandling. Överlevnaden i äggstockscancer har förbättrats genom centralisering till få centra med kirurger subspecialiserade i gynekologisk tumörkirurgi. Behandlingen av äggstockscancer centraliserades i Sverige 2012.

Syftet med min avhandling var att undersöka nya protein biomarkörer för deras potential att förbättra den diagnostiska förmågan av CA125 och HE4 (studie I och II), att jämföra äggstockscancerkirurgi på regionala sjukhus och tertiära centra efter centralisering (studie III) och att analysera incidens och överlevnad i EOC i Sverige sedan 1960-talet (studie IV).

Studie I: CA125, HE4, B7-H4 och suPAR analyserades i plasmaprover från 350 kvinnor med planerat operation för äggstockstumör. Plasmanivåer av CA125, HE4 och suPAR(II-III) ökade från godartade tumörer över borderline tumörer och EOC I till EOC II. B7-H4 var endast stegrat i EOC II. En kombination av CA125, HE4,

suPAR(II-III) och ålder visade bättre förmåga att skilja på godartade tumörer och cancer jämförd med Risk of Ovarian Malignancy Algorithm (ROMA), som kombinerar CA125, HE4 och menopausstatus, för premenopausala kvinnor. ROMA presterade bättre för postmenopausala kvinnor.

Vi korrelerade biomarkörnivåer med överlevnad efter diagnos. Kvinnor med höga värden av HE4, CA125 och suPAR(I) innan operation hade sämre överlevnad. SuPAR(I) var en oberoende markör för dålig överlevnad under första året efter diagnos, och mycket dålig prognos sågs för kvinnor över 75 år med hög suPAR(I).

Studie II: 177 inflammations- och cancerrelaterade biomarkörer analyserades i preoperative plasmaprover från 180 kvinnor med äggstockstumörer. HE4 var den bästa unika markören för att skilja mellan godartade tumörer och EOC. Kombinationer av HE4, CA125 och en ytterligare biomarkör jämfördes med en modell med HE4 och CA125. Ingen markör förbättrade modellen med HE4 och CA125 till en signifikant nivå.

Studie III: Data från Nationella kvalitetsregistret inom gynekologisk kirurgi (GynOp registret) 2013-15 analyserades. Av 1108 registrerade operationer för äggstockscancer utfördes 30 % på regionala sjukhus med mindre än 20 operationsfall per år. Fyra tertiära centra utförde mer än 25 operationer per år. Tertiära centra utförde mer omfattande kirurgi utan ökat antal allvarliga komplikationer jämförd med regionala sjukhus. Det fanns stora skillnader i patientselektion till primär operation och andelen patienter med komplett tumörresektion vid primär operation bland de tertiära centrarna.

Studie IV: Vi identifierade alla kvinnor med diagnos av epitelial äggstocks-, äggledar-, bukhinnecancer eller ospecificerad cancer i buk eller bäcken i det Svenska Cancerregistret 1960 - 2014. Åldersstandardiserad incidens och relativ överlevnad analyserades och tidstrendgrafer modellerades för ålder, tumörursprung och morfologi. Sedan 1980 har den åldersstandardiserade incidensen av EOC minskat i Sverige. Den relativa överlevnaden har förbättrats upp till 5 år efter diagnos från 1960 till 2014. 10-års överlevnaden har inte förbättrats sedan 1960.

Sammanfattningsvis framstår CA125 och HE4 som den bästa biomarkörkombination för bedömning av cancerrisk hos en kvinna med äggstockstumör. CA125, HE4 och suPAR(I) har potential som prognostiska markörer. Biomarkörer kan vara till hjälp i den preoperativa bedömningen, särskilt hos äldre kvinnor, vid beslut om primär kirurgi eller cellgiftbehandling.

Efter centralisering av den kirurgiska behandlingen för äggstockscancer i Sverige 2012 opereras många kvinnor fortsatt vid regionala sjukhus med få äggstockscancerfall per

år. Behandlingen för avancerad EOC skiljer sig åt betydligt mellan tertiära centra. Det verkar finnas ett behov av fortsatt centralisering samt ökat samarbete och utbyte mellan de tertiära centrarna för att säkra en likvärdig vård för patienter som drabbats av äggstockscancer.

Förbättrad kirurgisk och onkologisk behandling har förbättrat överlevnaden i EOC upp till 5 år efter diagnos, men långtidsöverlevnaden har inte förbättrats sedan 1960. Patienterna dör fortfarande i hög grad av sin sjukdom. För att kunna bota EOC måste sjukdomen hittas i tidigare stadier. Det finns ett stort behov av bättre diagnostiska metoder för tidig upptäckt av EOC.

List of abbreviations

ASA: American Society of Anesthesiologists

ASR: Age-standardised incidence rate

AUC: Area under the curve

B7-H4: B7 protein family homologue 4

BMI: Body mass index

BRCA1 and 2: Breast cancer susceptibility protein 1 and 2

CA125: Cancer antigen 125

CPH-I: Copenhagen Index

EOC: Epithelial ovarian cancer

FDA: The US Food and Drug Administration

FDR: False discovery rate

FIGO: Fédération Internationale de Gynécologie et d'Obstétrique

FSH: Follicle stimulating hormone

HE4: Human epididymis protein 4

HGSC: High-grade serous cancer

HRD: Homologous recombination defect

HRT: Hormonal replacement therapy

ICD: International classification of diseases

IDS: Interval debulking surgery

IOTA: International Ovarian Tumour Analysis group

LGSC: Low-grade serous cancer

LOH: Loss of heterozygosity

LR: Likelihood ratio

NACT: Neoadjuvant chemotherapy

NPV: Negative predictive value

OS: Overall survival

PARP: Poly (ADP-ribose) polymerase

PCR: Polymerase chain reaction

PDS: Primary debulking surgery

PEA: Proximity Extension Assay

PFS: Progression-free survival

PPV: Positive predictive value

RH: Regional hospital

RMI: Risk of malignancy index

ROCA: Risk of ovarian cancer algorithm

ROC: Receiver operating characteristic

ROMA: Risk of ovarian malignancy algorithm

RRSO: Risk-reducing salpingo-oophorectomy

RS: Relative survival

SCR: Swedish Cancer Registry

SEERS: Surveillance, Epidemiology, and End Results programme

STIC: Serous tubal intraepithelial carcinoma

suPAR: soluble urokinase plasminogen activator receptor

TC: Tertiary centre

TVS: Transvaginal ultrasound scan

VEGF: Vascular endothelial growth factor

WFDC2: WAP four-disulfide core domain 2 gene

WHO: World Health Organization

Introduction

Ovarian cancer

Epidemiology

Incidence

Ovarian cancer is the eighth most common female cancer worldwide and the most lethal of the gynaecologic malignancies. In 2018 an estimated 295,414 women were diagnosed with ovarian cancer and 184,799 women died of the disease. Large variations in incidence are found between countries and continents, with the highest incidence in central and Eastern Europe (Fig. 1) (Bray et al. 2018).

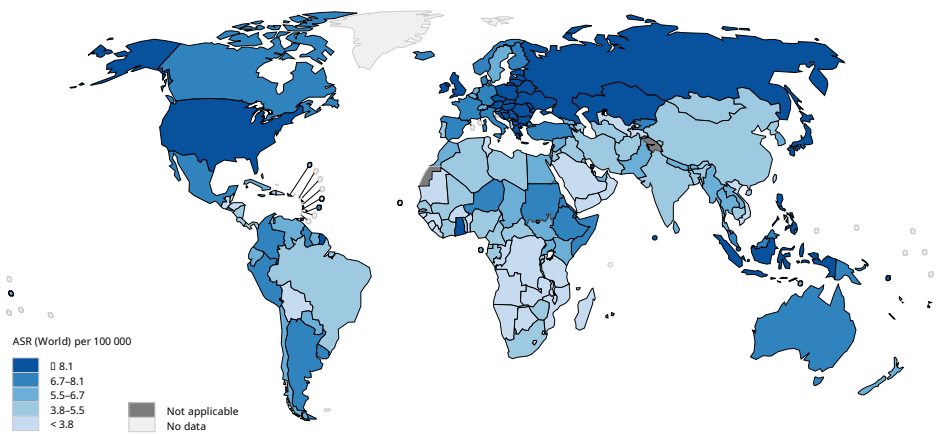

Figure 1. Worldwide variations in ovarian cancer incidence. Source: GLOBOCAN. Estimated ovarian cancer incidence in the world 2018, age-standardised incidence rates.

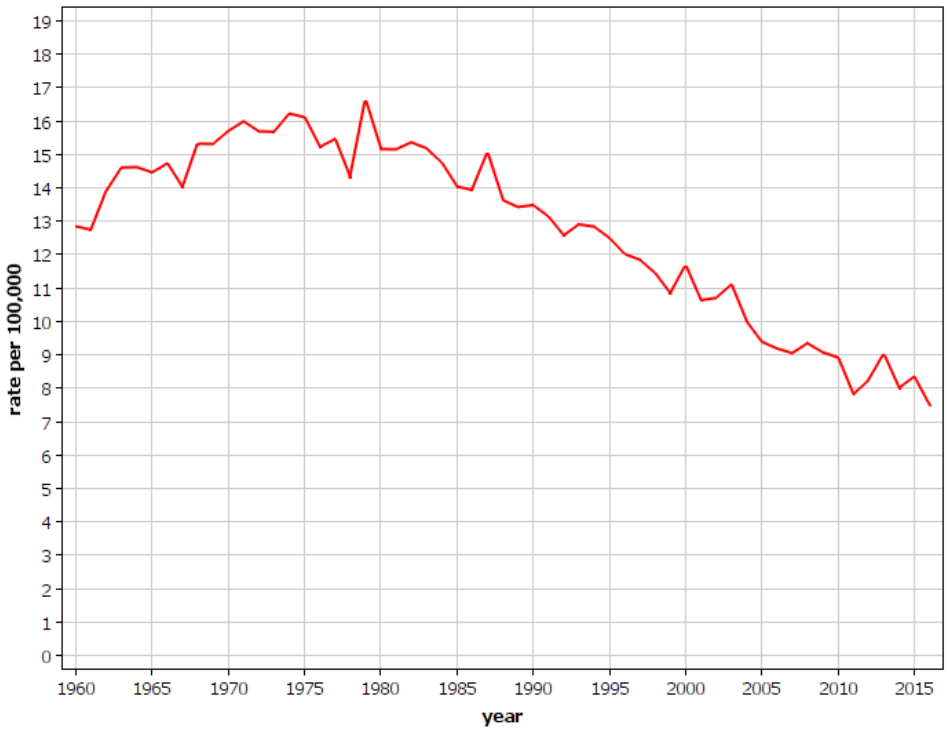

Figure 2. The incidence in ovarian cancer has declined in Sweden since 1980. Source: NORDCAN. Ovarian cancer incidence in Sweden 1958-2016, age-standardised incidence rates.

The age-standardised incidence rate (ASR) is declining in many countries of Northern Europe and North America, while incidence is rising in other parts of the world (Coburn et al. 2017). Since 1980, ovarian cancer ASR has declined in Sweden (Fig. 2) (Danckert B et al. 2019).

The reasons for the striking decline in ovarian cancer incidence in many developed countries have been discussed widely. A major contributing factor is believed to be the widespread use of the contraceptive pill, introduced in the 1960s (Beral et al. 2008). Also, the use of hormonal replacement therapy (HRT) has decreased since the large Women's Health Initiative trial in 2002 reported the risk of breast cancer and cardiovascular disease to be increased in postmenopausal women on HRT. The decline in HRT use after 2002 has been found to correlate with an accelerated decline in ovarian cancer incidence rates (Rossouw et al. 2002, Yang et al. 2013).

Another factor contributing to the declining incidence is the introduction of different tumour classification criteria. Borderline ovarian tumours were previously often classified as low-grade ovarian cancer. Since the 1970s these tumours have been

recognised as a separate category in the FIGO and WHO classifications (Hauptmann et al. 2017). Since the 1960s the incidence of borderline tumours in Sweden has increased dramatically, from 8% to 24% of all primary ovarian neoplasms in the period 2000-2005, coinciding with the decline in ovarian cancer incidence (Skírnisdóttir et al. 2008, Kalapotharakos et al. 2016).

Ovarian cancer primarily affects postmenopausal women. In Sweden the majority of patients are diagnosed at 65-70 years of age. The lifetime risk for a Swedish woman to develop ovarian cancer is 1 in 80 (Danckert B et al. 2019).

Survival

If diagnosed at an early stage, ovarian cancer has a favourable prognosis. The five-year survival is 92% for localised disease (FIGO stage IA-B). Sadly, the majority of patients (60%) are diagnosed in late stages (FIGO III-IV), where cure is rarely possible, resulting in a five-year survival of 29% (Siegel, Miller and Jemal 2019). Modern-day therapy has prolonged life after diagnosis. In Sweden the five-year survival in ovarian cancer was 30% in the period 1967-1971, increasing to 49% for 2012-16 (Fig. 3) (Danckert B et al. 2019).

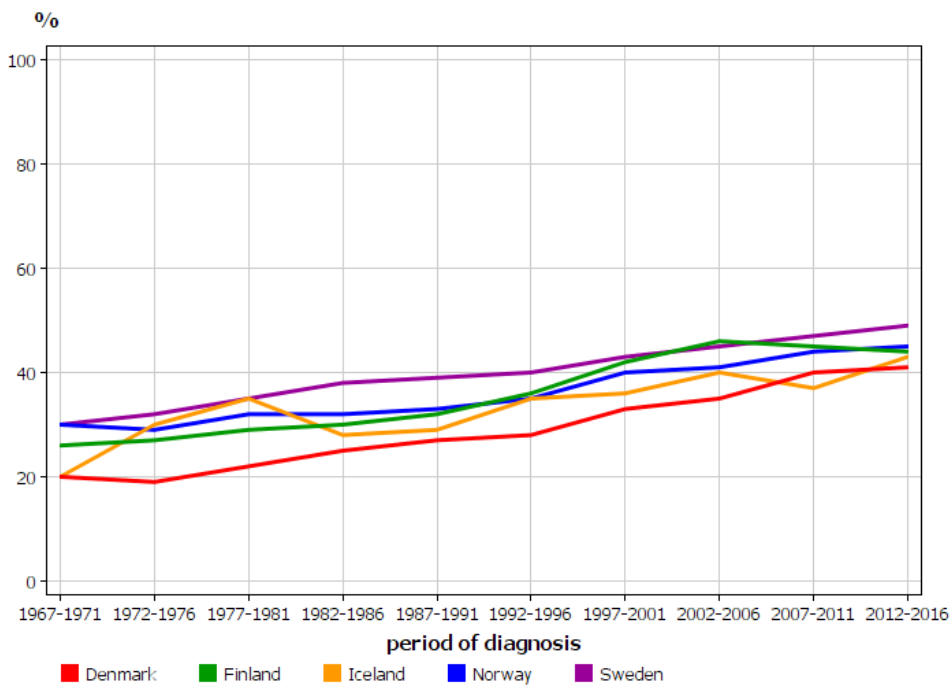

Figure 3. The five-year survival in ovarian cancer has improved since the 1960s. Source: NORDCAN. Five-year age-standardised relative survival in ovarian cancer in the Nordic countries 1967-2016.

Aetiology

Ovarian cancer is a heterogeneous disease, with multifactorial aetiology. The main focus of this thesis is epithelial ovarian cancer (EOC), the most common and lethal type of ovarian cancer. EOC accounts for 90% of ovarian cancer cases, while germ cell and sex cord stromal tumours constitute the remaining 10%. The five main histological subgroups of EOC are high-grade serous cancer (HGSC) (70%), endometrioid cancer (10%), mucinous cancer (3%), clear cell cancer (10%) and low-grade serous cancer (LGSC) (<5%) (Prat 2012).

Borderline tumours constitute a separate entity of epithelial ovarian neoplasms, with higher epithelial proliferation than is seen in benign tumours and a variable degree of nuclear atypia, but no stromal invasion as is found in epithelial carcinomas. Serous (50%) and mucinous tumours (40%) are the most common. Less than 3% of borderline tumours progress to invasive carcinoma. The majority of borderline tumours are diagnosed in early-stage (FIGO I) and the prognosis is excellent, with a five-year survival of 97% (Kalapotharakos et al. 2016, Prat 2017, Hauptmann et al. 2017).

Historically, two main hypotheses have been proposed for the pathogenesis of EOC; the "incessant ovulation hypothesis" and the "gonadotropin hypothesis".

The incessant ovulation hypothesis

The repeated minor trauma to the ovarian surface epithelium and the exposure of the epithelium to oestrogen-rich follicular fluid by repeated ovulation is suggested to induce epithelial neoplasia. Accordingly, a high number of ovulations increases the risk of EOC, while inhibition of ovulation will have a protective effect (Fathalla 1971). A recent study by the Ovarian Cancer Cohort Consortium found a linear relationship between lifetime ovulatory cycles and ovarian cancer risk (Trabert et al. 2020).

The gonadotropin hypothesis

Ovarian epithelial neoplasia is proposed to develop from ovarian inclusion cysts in response to high levels of gonadotropins or oestrogens. In support of this hypothesis, the ovarian cancer risk is increased after menopause, when gonadotropin levels are high, and an increased cancer risk is found in women exposed to high levels of oestrogen (Cramer and Welch 1983).

Both these theories have assumed an ovarian origin for EOC. However, in the last two decades growing evidence has supported the view that the origin of the majority of HGSC is serous tubal intraepithelial carcinomas (STICs) in the fallopian tubes (Piek et al. 2001, Medeiros et al. 2006, Kindelberger et al. 2007).

EOC type I and II

A dualistic model has been proposed by Kurman and Shih, categorising epithelial ovarian cancer into two main groups based on origin and pathogenesis: EOC types I (25%) and II (75%) (Table 1). Type I tumours develop step-wise from benign precursors (cystadenomas or endometriosis) to borderline tumours and then to invasive carcinomas. They are slow-growing, genetically stable and often confined to the ovary at diagnosis, with good prognosis. However, in advanced stages, the prognosis is poor, due to poor response to chemotherapy. Mutations can be seen in KRAS, BRAF, ERBB2, PTEN, PIK3CA, ARID1A and CTNNB1 and in the DNA mismatch repair (MMR) genes. Type I tumours account for 10% of deaths in EOC. Most type II tumours evolve from STICs, with secondary spread to the ovaries and peritoneum. These are high-grade, aggressive tumours, in most cases disseminated at diagnosis and cause 90% of EOC deaths. Type II tumours have high chromosomal instability with TP53 mutations. Homologous recombination defects (HRD) including BRCA1/2 mutations are common. (Shih and Kurman 2004, Kurman and Shih 2016).

Table 1. Epithelial ovarian cancer types I and II. LGSC: Low-grade serous carcinoma, HGSC: High-grade serous carcinoma (Kurman and Shih 2016).

EOC	**Type I**				**Type II**
Origin:	Endometriosis	Fallopian tube	Germ cell	Transitional epithelium	Fallopian tube
Subtype:	Endometrioid carcinoma Clear cell carcinoma Seromucinous carcinoma	LGSC	Mucinous carcinoma	Mucinous carcinoma Brenner cell tumours	HGSC Carcinosarcoma Undifferentiated carcinoma

Protective factors

Parity and breastfeeding are well-established protective factors for ovarian cancer (Luan et al. 2013, Gaitskell et al. 2018). Use of oral contraceptives reduces the risk of ovarian cancer, with greater risk reduction the longer the use, and a persistent risk reduction is found up to 30 years after use (Beral et al. 2008). The effect of other contraceptives has been less investigated, including the increasingly widespread levonorgestrel intrauterine system (LNG-IUS). Recent studies report a strong risk reduction for ovarian and endometrial cancer in LNG-IUS users (Soini et al. 2014, Jareid et al. 2018). Prophylactic oophorectomy is a well-known risk-reducing measure in high-risk populations with familial ovarian cancer (Domchek et al. 2010), but even hysterectomy, tubal ligation or salpingectomy confers a reduced risk of ovarian cancer, with the highest risk reduction found for salpingectomy (Falconer et al. 2015).

Risk factors

Endometriosis is a risk factor for endometrioid and clear cell ovarian cancer (Borgfeldt and Andolf 2004, Kim et al. 2014). The use of hormonal contraceptives seems to confer a larger protective effect in endometriosis patients. Modugno et al. found a risk reduction from long-term oral contraception use of up to 80% for ovarian cancer in women with endometriosis (Modugno et al. 2004). Infertility, and the use of assisted reproductive technology, are associated with an increased risk of invasive ovarian cancer and borderline ovarian tumours (Lundberg et al. 2019). Pelvic inflammatory disease is also a risk factor for borderline tumours (Rasmussen et al. 2017), and past Chlamydia infection has recently been associated with ovarian cancer (Trabert et al. 2019, Idahl et al. 2020). Obesity increases the risk of ovarian borderline tumours and EOC type 1 (Collaborative Group on Epidemiological Studies of Ovarian Cancer 2012, Olsen et al. 2013). Use of HRT in menopausal women increases ovarian cancer risk. The risk increases with duration of HRT use and persists for up to a decade after long-term use (>5 years) (Beral et al. 2015). Smoking appears to be a risk factor for mucinous tumours only (Beral et al. 2012).

Hereditary factors

15% to 25% of EOCs are hereditary. Around 80% are associated with mutations in the BRCA1 or BRCA2 tumour suppressor genes (hereditary breast and ovarian cancer syndrome). Mutations in the DNA mismatch repair genes linked to Lynch syndrome (Hereditary non-polyposis colorectal cancer) were previously the most common findings in non-BRCA mutated hereditary ovarian cancer. However, a growing number of new germline mutations have been found to contribute to increased ovarian cancer risk in recent years (Song et al. 2015, Norquist et al. 2016, Tung et al. 2016, Harter et al. 2017).

For women with the BRCA1 mutation the lifetime risk of EOC is estimated to be 40% to 50% and for women with the BRCA2 mutation the risk is 15% to 30%, while the risk of breast cancer is considerably higher, at 50% to 80%. Mutations in BRCA1 are more common than BRCA2 mutations. Age at diagnosis is lower and the prognosis more favourable for hereditary ovarian cancer compared to sporadic cases (Boyd et al. 2000, Prat, Ribé and Gallardo 2005, Kuchenbaecker et al. 2017). The proteins encoded by the BRCA1 and 2 genes have important functions in DNA damage repair. Loss of BRCA1 or 2 function leads to genomic instability due to HRD and increased risk of malignancy (O'Connor 2015). Patients with germline BRCA1 or 2 mutations inherit one mutated allele. Somatic loss of the other allele (loss of heterozygosity, LOH) is needed for BRCA inactivation and carcinogenesis (Prat et al. 2005).

In Sweden, genetic screening of EOC patients and their families has been implemented since 2017. Oral contraceptives lower the risk of ovarian cancer without increased risk of breast cancer in healthy BRCA mutation carriers (Moorman et al. 2013). Risk-reducing mastectomy and salpingo-oophorectomy (RRSO) are recommended in healthy carriers, the latter after child-bearing is completed, preferably before 40 years of age (Hartmann and Lindor 2016). RRSO confers a risk reduction of up to 80% for ovarian cancer and up to 60% for breast cancer in BRCA1 and BRCA2 carriers (Domchek et al. 2010, Finch et al. 2014). In recent years, another strategy, interval salpingectomy with delayed oophorectomy (ISDO) has been suggested as an alternative to RRSO in high-risk women wishing to delay menopause. However, studies are lacking regarding the effects of ISDO on cancer risk and quality of life (Long Roche et al. 2017).

Women with Lynch syndrome have an increased risk of colorectal cancer (30% to 75%), as well as a range of other cancers including endometrial and ovarian cancer. The lifetime risk of endometrial cancer is 25% to 60% while for ovarian cancer it is 5% to 20% (Lynch et al. 2009, Goodenberger et al. 2016). Close surveillance with ultrasound and endometrial biopsies from a young age and prophylactic hystero-salpingo-oophorectomy after child-bearing is recommended (Tzortzatos et al. 2015, Goodenberger et al. 2016).

Ovarian cancer staging

In recognition of the extra-ovarian origin of a high proportion of EOC, the 2014 International Federation of Gynaecology and Obstetrics (FIGO) staging is common for ovarian, fallopian tube and primary peritoneal cancers (Prat 2014).

Table 2. 2014 FIGO classification of ovarian cancer (Prat 2014).

Stage I. Tumour confined to ovaries or fallopian tube(s)
IA: tumour limited to one ovary (capsule intact) or fallopian tube; no tumour on ovarian or fallopian tube surface; no malignant cells in the ascites or peritoneal washings **IB:** tumour limited to both ovaries (capsules intact) or fallopian tubes; no tumour on ovarian or fallopian tube surface; no malignant cells in the ascites or peritoneal washings **IC:** tumour limited to one or both ovaries or fallopian tubes, with any of the following: **IC1:** surgical spill **IC2:** capsule ruptured before surgery or tumour on ovarian or fallopian tube surface **IC3:** malignant cells in the ascites or peritoneal washings
Stage II. Tumour involves one or both ovaries or fallopian tubes with pelvic extension (below pelvic brim) or primary peritoneal cancer
IIA: extension and/or implants on uterus and/or fallopian tubes and/or ovaries **IIB:** extension to other pelvic intraperitoneal tissues
Stage III. Tumour involves one or both ovaries or fallopian tubes, or primary peritoneal cancer, with cytologically or histologically confirmed spread to the peritoneum outside the pelvis and/or metastasis to the retroperitoneal lymph nodes
IIIA1: positive retroperitoneal lymph nodes only (cytologically or histologically proven): IIIA1(i) Metastasis up to 10 mm in greatest dimension IIIA1(ii) Metastasis more than 10 mm in greatest dimension **IIIA2:** microscopic extra-pelvic (above the pelvic brim) peritoneal involvement with or without positive retroperitoneal lymph nodes **IIIB:** macroscopic peritoneal metastasis beyond the pelvis up to 2 cm in greatest dimension, with or without metastasis to the retroperitoneal lymph nodes **IIIC:** macroscopic peritoneal metastasis beyond the pelvis more than 2 cm in greatest dimension, with or without metastasis to the retroperitoneal lymph nodes (includes extension of tumour to capsule of liver and spleen without parenchymal involvement of either organ)
Stage IV. Distant metastasis excluding peritoneal metastases
IVA: pleural effusion with positive cytology **IVB:** parenchymal metastases and metastases to extra-abdominal organs (including inguinal lymph nodes and lymph nodes outside the abdominal cavity)

Symptoms

Referred to as "The silent killer" or "The whispering disease", ovarian cancer in most cases presents with vague, unspecific symptoms, causing delay in seeking health care. Common symptoms are abdominal swelling, pain, indigestion, altered bowel habits, urinary symptoms and fatigue, misattributed by many women and doctors to benign

causes including stress, menstruation, menopause, or irritable bowel syndrome. While these symptoms are indeed very common in women attending general practice for benign conditions, symptoms tend to have a more recent onset, are more severe and frequent, and often co-exist in women later diagnosed with ovarian cancer (Goff et al. 2004). Still, many general practitioners will attribute symptoms to non-cancer causes initially, causing further diagnostic delay (Evans, Ziebland and McPherson 2007, Seibaek et al. 2011). Previously believed to be asymptomatic in early stages, only 7% to 10% of patients diagnosed with early-stage ovarian cancer had no symptoms prior to diagnosis (Bankhead, Kehoe and Austoker 2005).

Screening for ovarian cancer

Due to late diagnosis, the prognosis is poor for most ovarian cancer patients. If more cancers could be detected and treated in early stages, survival is expected to improve radically. A screening strategy for ovarian cancer has been sought for decades. Given the low prevalence of ovarian cancer in the general population (around 1 in 2500 postmenopausal women), a sensitivity of at least 75% and a very high specificity of 99.6%, corresponding to a PPV of 10%, is recommended in order for a screening method to be acceptable in society. This corresponds to one diagnosis of ovarian cancer out of 10 women with a positive screening test. While neither transvaginal ultrasound scan (TVS) nor the plasma biomarker CA125 fulfil these criteria as individual screening tests, performance is markedly improved when the two are combined (Jacobs and Menon 2004, Yang, Lu and Bast 2017). Strategies combining TVS and CA125 have been tested in a range of screening trials.

In recent years, two large-scale prospective population screening studies in postmenopausal women, the PLCOS (Prostata, Lung, Colorectal and Ovarian cancer Screening) and UKCTOCS (United Kingdom Collaborative Trial on Ovarian Cancer Screening) trials, have been unable to show a significant decrease in ovarian cancer mortality from annual screening with CA125 and TVS or ROCA (Risk of Ovarian Cancer Algorithm, described in detail in the section on protein biomarkers in ovarian cancer). However, in a subgroup analysis of the UKCTOCS a 20% reduction in mortality was found among incident cancer cases for women screened with ROCA, and a stage-shift was observed for screening-detected cases in the ROCA group. Continued follow-up for the UKCTOCS may strengthen these findings. The number of women having surgery for screening-detected, suspected malignancy with benign findings was 1% in the ROCA group, corresponding to a PPV of 30.3% (Buys et al. 2011, Jacobs et al. 2016).

In conclusion, while some promising data have been reported in the UKCTOCS, screening for ovarian cancer has so far not been shown to decrease mortality and is not recommended (Henderson, Webber and Sawaya 2018).

Ovarian cancer diagnostics

While waiting for an effective screening strategy to arrive, considerable efforts have been made to improve the initial assessment of women with suspicion of ovarian cancer.

Standardised ovarian cancer care pathway

In Sweden, national fast tracks for the diagnosis and treatment of cancer have been implemented since 2015. The standardised ovarian cancer care pathway ensures fast referral from primary care to gynaecological assessment of women with symptoms causing suspicion of ovarian cancer (within 10 days from referral). If suspicion persists after gynaecological assessment, the patient is referred to a tertiary ovarian cancer care centre with the aim of surgical treatment within 24 days or oncological treatment within 22 days from referral (RCC 2015).

Ultrasound in ovarian cancer diagnostics

The majority of clinical guidelines, including the Swedish ones, recommend ultrasound and/or CA125 to be part of the initial assessment of women with suspected ovarian cancer (Funston et al. 2019). The transvaginal ultrasound scan (TVS) is superior to transabdominal ultrasound for visualisation of the ovaries. Sensitivity is high for identification of malignant tumours. However, due to a high prevalence of benign ovarian tumours among healthy women, the specificity and PPV are relatively low, with a considerable number of women with an adnexal mass assigned to diagnostic surgery with benign findings (Bailey et al. 1998, van Nagell et al. 2000, Jokubkiene, Sladkevicius and Valentin 2014). Furthermore, the quality of ultrasound diagnostics is highly dependent on the skills and experience of the examiner (Van Holsbeke et al. 2010).

The IOTA (International Ovarian Tumour Analysis) group is dedicated to improving and standardising the ultrasound assessment of adnexal tumours (Timmerman et al. 2000). Several strategies to improve the diagnostic performance of ultrasound in the hands of less experienced examiners have been introduced by the IOTA group, including the LR1 and LR2 logistic regression models (Timmerman et al. 2005), the Simple Rules (Timmerman et al. 2008, Timmerman et al. 2016) and the Simple Descriptors (Ameye et al. 2012). The IOTA models have been found to perform with

comparable diagnostic performance when used by examiners with varied ultrasound experience (Sayasneh et al. 2013b, Sayasneh et al. 2013a, Nunes et al. 2013). The use of the IOTA strategies in the assessment of adnexal mass is strongly encouraged, especially for non-expert examiners.

Protein biomarkers in ovarian cancer

Clearly, the effort to improve survival in ovarian cancer is challenged by inadequate diagnostic tools, with most patients diagnosed too late for the disease to be curable, and many women with benign adnexal tumours subjected to unnecessary surgical interventions, with the risk of morbidity and compromised future fertility. Much hope and research has been placed on DNA, RNA, protein or metabolite-based biomarkers in biological fluids (serum, plasma, urine, ascites, cyst fluid) to improve cancer diagnostics. Despite extensive research, few biomarkers have been implemented in clinical practice (Hanash 2011, Carvalho et al. 2019). A large range of protein biomarkers have been investigated for their potential in screening for ovarian cancer, in the risk assessment of ovarian tumours, or as prognostic markers in ovarian cancer treatment. To date, none have outperformed the Cancer Antigen 125 (CA125) and Human Epididymis protein 4 (HE4).

CA125

First described 40 years ago, the Cancer Antigen 125 is a peptide epitope on a high-weight glycoprotein, subsequently identified as Mucin 16 (MUC16), expressed and secreted by epithelial ovarian tumours and other tissues of Müllerian origin (the fallopian tubes, endometrium and endocervix) as well as coelomic epithelia including the peritoneum, pleura and pericardium. CA125 is elevated in > 80% of patients with EOC, although in early-stage EOC (FIGO stage I), only 50% of the patients have elevated CA125. CA125 expression varies between tumour histological subtypes, with low expression levels in mucinous tumours (Bast et al. 1981, Jacobs and Bast 1989, Yin and Lloyd 2001). CA125 can be elevated in several other malignancies including breast and lung cancer (Sjövall, Nilsson and Einhorn 2002). Benign ovarian tumours as well as many other benign conditions may present with elevated levels of CA125, including endometriosis, pelvic inflammatory disease, early pregnancy, ascites of all causes, and congestive heart failure (Jacobs and Bast 1989). Due to these limitations to its sensitivity and specificity, CA125 as a single test is inadequate for ovarian cancer diagnostics (Jacobs and Menon 2004, Medeiros et al. 2009). To improve performance,

CA125 testing has been combined with TVS, serial CA125 assessment over time, and with other biomarkers.

The mucin expressing CA125, MUC16, has important roles in tumour pathogenesis. MUC16 contributes to ovarian tumour growth and metastasis, indicating CA125/MUC16 is not only a clinically useful biomarker but also an attractive target for therapeutic strategies for epithelial ovarian cancer. However, while in vitro and in vivo studies have shown promising results, clinical studies are few and have shown limited benefits so far (Thériault et al. 2011, Felder et al. 2014, Aithal et al. 2018).

HE4

Human Epididymis protein 4 is a secreted glycoprotein product of the WFDC2 (WAP four-disulfide core domain 2) gene. In normal tissues, HE4 expression is restricted to the epididymis of the male reproductive tract, the Müllerian tissues of the female reproductive tract and respiratory epithelium. HE4 is overexpressed by the majority of serous and endometrioid EOC, with lower expression in clear cell EOC and no expression in mucinous EOC. High levels of HE4 are also found in endometrial cancer, with lower levels reported in cancers of non-ovarian origin, including lung, breast, gastrointestinal and uroepithelial carcinoma (Drapkin et al. 2005, Galgano, Hampton and Frierson 2006, Hertlein et al. 2012). HE4 is less frequently increased in benign gynaecological and non-gynaecological disease than CA125. However, HE4 increases with age, and high levels are seen in renal failure (Hellström et al. 2003, Moore et al. 2012, Hertlein et al. 2012). HE4 performs comparably to CA125 in distinguishing ovarian cancer from healthy controls but has higher sensitivity for discrimination between malignant and non-malignant disease than CA125, especially in early-stage EOC and in premenopausal women (Hellström et al. 2003, Moore et al. 2008). Combining CA125 and HE4 improves sensitivity and specificity for ovarian cancer compared to CA125 or HE4 alone (Moore et al. 2008).

In recent years, HE4, like CA125, has been found to contribute to cancer progress. Overexpression of HE4 promotes tumour proliferation, metastasis and chemo-resistance, while downregulation suppresses tumour growth (Zhu et al. 2013, Wang et al. 2019, Zhu et al. 2016, Ribeiro et al. 2016). Inhibition of HE4 may prove an efficient future strategy in EOC. To date, clinical studies are lacking (James, Chichester and Ribeiro 2018).

Several algorithms have been introduced to improve the diagnostic performance of CA125 and HE4 over the years.

Screening

Risk of Ovarian Cancer Algorithm (ROCA)

The ROCA was developed by Steven Skates and colleagues for ovarian cancer screening. The ROCA compares a woman's individual CA125 profile, based on longitudinal changes in CA125, to CA125 profiles for women with ovarian cancer and controls. The closer the profile to the "change-point" profiles of women who developed ovarian cancer, the greater the risk of ovarian cancer. An intermediate ROCA risk triggers a CA125 test in three months and the risk is recalculated. Elevated ROCA risk triggers referral to TVS (Skates 2012). Population screening with the ROCA was tested in the UKCTOCS trial with promising results. Extended follow-up data are expected in 2021 (Jacobs et al. 2016).

Diagnostics

Risk of Malignancy Index (RMI)

The RMI algorithm, based on CA125, ultrasound score and menopause status, was introduced by Jacobs et al. in the 1990s and is recommended for the initial assessment of women with suspected ovarian cancer in the Swedish national guidelines (Jacobs et al. 1990, RCC 2019). The RMI discriminated between benign and malignant ovarian tumours with 85% sensitivity, and 97% specificity at the recommended cut-off of RMI 200 in the original study by Jacobs et al. (Jacobs et al. 1990). Higher sensitivity (92%) is reported for the use of the RMI for preoperative assessment in a tertiary setting when ultrasound is performed by experts (Hakansson et al. 2012). However, the need for ultrasound evaluation limits the use of the RMI at primary care level.

Table 3. Risk of Malignancy Index (Jacobs et al. 1990). https://www.mdcalc.com/risk-malignancy-index-rmi-ovarian-cancer

RMI = M x U x CA-125	
M score: M = 1 if premenopausal M = 3 if postmenopausal	
U score: U = 0 if no ultrasound feature U = 1 if 1 feature U = 3 if more than 1 feature	Ultrasound features: Multilocularity Presence of solid areas Bilaterality Ascites Metastases
CA-125 in U/mL	
Recommended cut-off RMI > 200 to indicate high risk of ovarian malignancy	

Risk of Ovarian Malignancy Algorithm (ROMA)

In 2009 Moore et al. introduced the ROMA, incorporating CA125, HE4 and menopause status, dispensing with the need for ultrasound evaluation. The ROMA was shown to discriminate between benign and malignant ovarian tumour with a sensitivity of 94% and NPV of 99% at a set specificity of 75% (Moore et al. 2009). In their study comparing the ROMA and the RMI in 2010, Moore et al. found better performance for the ROMA compared to the RMI, although these findings have been questioned by subsequent studies (Moore et al. 2010, Karlsen et al. 2012, Van Gorp et al. 2012, Yanaranop et al. 2017). Both algorithms have decreased sensitivity and specificity in early stages of EOC and in premenopausal women (Lennox et al. 2015).

Table 4. Risk of Ovarian Malignancy Algorithm (Moore et al. 2009). https://diagnostics.roche.com/global/en/article-listing/roma-calculator.html

Risk of Ovarian Malignancy Algorithm (ROMA)
Premenopausal: Predictive Index (PI) = – 12.0 + 2.38∗ln(HE4) + 0.0626∗ln(CA 125)
Postmenopausal: Predictive Index (PI) = – 8.09 + 1.04∗ln(HE4) + 0.732∗ln(CA 125)
Predicted Probability (PP) = exp(PI) / [1 + exp(PI)]
Recommended cut-off to indicate high risk of ovarian malignancy: PP > 13.1% for premenopausal women PP > 27.7% for postmenopausal women

The Copenhagen Index (CPH-I)

The CPH-I is a modified version of the ROMA, combining CA125, HE4 and age. The CPH-I was found to perform as well as the RMI and the ROMA in an international multicentre study (Karlsen et al. 2015). The substitution of menopause status for age minimises the risk of bias from the subjective assessment of menopause, and with no need for ultrasound the CPH-I may prove to be the clinically most useful algorithm of the three in the primary care setting.

OVA1 and Overa

The OVA1 test (Vermillion, Inc.), was introduced in the US in 2009 for the preoperative risk assessment of women scheduled for surgery for ovarian tumour. The OVA1 measures CA125, transferrin, transthyretin, apolipoprotein AI, and beta2 microglobulin in a multivariate assay, generating a malignancy risk score with a pre- and postmenopausal cut-off to indicate high or low risk of malignancy. The OVA1 performs with very high sensitivity for ovarian cancer, including all morphologies, early-stage disease and premenopausal women, compared to CA125 or clinical assessment alone, but at the cost of low specificity and PPV (Ueland et al. 2011, Bristow

et al. 2013, Longoria et al. 2014). A second generation of the OVA1, Overa (Vermillion, Inc.), was introduced in 2016. In Overa, transthyretin and beta2 microglobulin were substituted for HE4 and FSH, with maintained sensitivity and improved specificity compared to the OVA1. The inclusion of FSH replaced the need for information on menopause status (Coleman et al. 2016, Ueland 2017). External validation studies comparing the performance of the OVA1 or Overa with the ROMA or the RMI are lacking.

Ultrasound vs. biomarker algorithms

Ultrasound outperforms both the RMI and ROMA in the risk assessment of ovarian tumours (Van Gorp et al. 2012, Kaijser et al. 2014, Testa et al. 2014).

The latest IOTA model, the ADNEX (Assessment of Different NEoplasias in the adneXa), combines three clinical (including CA125) and six ultrasound markers to discriminate between benign, borderline, early- and late-stage malignant ovarian tumours and metastatic ovarian tumours, and also presents an overall risk of malignancy. http://www.iotagroup.org/adnexmodel/. Sensitivity is very high, 96.5%, with specificity 71.3% for overall discrimination between benign and malignant tumours in the hands of ultrasound specialists (Van Calster et al. 2014).

In the preoperative assessment of suspected cancer, high sensitivity is preferable to high specificity. The IOTA ultrasound-based models are superior in this regard. However, the ultrasound competence needed to apply these models is often limited to the tertiary care level. Biomarker tests are accessible in a primary care setting and can add improved specificity, to avoid over-diagnosis and unnecessary treatment of benign tumours.

Prognostic biomarkers

A prognostic biomarker can be defined as a biomarker used to identify the likelihood of a clinical event, disease recurrence or progression in patients who have the disease or medical condition of interest (FDA-NIH 2016). The only biomarkers currently approved by the FDA for the monitoring of treatment response and recurrence in EOC are CA125 and HE4.

CA125

While studies differ regarding the prognostic value of CA125 levels at diagnosis (Nagele et al. 1995, Markman et al. 2006, Zorn et al. 2009, Bandiera et al. 2011, Furrer et al. 2019), a decrease in CA125 levels indicates a response to chemotherapy and is prognostic for survival (Bast et al. 1983, Högberg and Kågedal 1990, Rustin et al. 1996a). The use of sequential CA125 as an indicator for treatment response is

recommended by the European Group on Tumour Markers, and by the Swedish national guidelines (Sölétormos et al. 2016, RCC 2019). In patients with normalised CA125 levels after primary chemotherapy, a rise in CA125 to double the upper limit of normal predicts tumour relapse, with a lead time of up to several months before clinical relapse, and in patients where CA125 never normalises, a doubling of the nadir value indicates progression (Rustin et al. 1996b, Rustin et al. 2001). However, a randomised trial comparing early treatment of relapse, based on a rise in CA125 only, with delayed treatment, at clinical relapse, did not find a survival benefit from early second-line treatment (Rustin et al. 2010). CA125 measurements should therefore not be offered as part of routine monitoring, but may be considered in patients eligible for clinical trials or secondary surgery at recurrence (Fleming et al. 2011). The Gynaecological Cancer Intergroup (GCIG) recommends the combination of CA125 with the radiological RECIST criteria (Response Evaluation Criteria in Solid Tumours) to define response and progress in ovarian cancer trials (Rustin et al. 2011, Sölétormos et al. 2016).

Preoperative CA125 levels have been suggested to predict the outcome of primary debulking surgery (PDS) in some studies, while others report no association (Chi et al. 2009, Vorgias et al. 2009). A meta-analysis from 2010 concluded that CA125 lacks the ability to accurately predict cytoreduction outcome. However, CA125 > 500 mU/L is a strong risk factor for suboptimal cytoreduction (Kang et al. 2010).

HE4

High HE4 levels at diagnosis predict poor prognosis in EOC (Bandiera et al. 2011, Paek et al. 2011, Kalapotharakos et al. 2012, Trudel et al. 2012, Furrer et al. 2019). Braicu et al. found high preoperative HE4 to be correlated with poor surgical outcome (residual tumour mass) and platinum resistance in primary treatment. The combination of CA125 and HE4 improved the prediction of surgical outcome compared to CA125 or HE4 alone (Braicu et al. 2013). Karlsen et al. 2016 found promising results for the combination of HE4 with age and performance status in the Cancer Ovarii Non-invasive Assessment of Treatment Strategy (CONATS) index, for the prediction of complete cytoreduction at PDS in advanced EOC (Karlsen et al. 2016).

Vallius et al. found HE4 and CA125 nadir values during postoperative chemotherapy to correspond to primary therapy outcome and PFS, and the combination of HE4 and CA125 to predict outcome better than either alone. (Vallius et al. 2017). Schummer et al. found HE4 predicted recurrence with a greater lead time than CA125 and was the only biomarker to rise before recurrence in a subset of patients, suggesting the addition of HE4 to CA125 in the monitoring of EOC recurrence (Schummer. et al. 2012) A

recent study found elevated HE4 levels to be a marker of recurrence in a small group of patients with CA125 negative tumours at diagnosis, indicating HE4 is of value for monitoring response and recurrence in this patient group (Plotti et al. 2019).

In summary, combining HE4 and CA125 holds promise for improved preoperative assessment, evaluation of treatment response and monitoring of recurrence in EOC. However, results vary between studies. Most of the above referred studies are small and the findings need further validation.

Proteomics

Despite the passage of four decades since discovery, CA125 continues to stand out as the single-best biomarker for ovarian cancer (Cramer et al. 2011, Terry et al. 2016). Much research has been focused on the search for additional biomarkers to improve the performance of CA125 (Nolen and Lokshin 2013, Ueland 2017). Adding to the difficulties in identifying biomarkers with high sensitivity and specificity for ovarian cancer is the heterogeneity of the disease, with different EOC subtypes expressing different patterns of biomarkers (Köbel et al. 2008, Tian et al. 2011, Wallstrom, Anderson and LaBaer 2013). The use of multiple protein panels that include markers specific to each subtype seems a promising strategy to improve diagnostic performance.

The molecular biology research field of proteomics (the large-scale study of proteins and proteomes) has provided new techniques for multiplex protein analysis in the search for candidate biomarkers. Common techniques for protein biomarker discovery include protein microarray and mass spectrometry, while immunoassays are commonly used for validation (Elzek and Rodland 2015, Carvalho et al. 2019). The OVA1 and Overa tests, described above, are the first examples of multivariate assays approved for clinical use in ovarian cancer diagnostics, incorporating protein biomarkers identified through serum proteomics using mass spectrometry (Zhang and Chan 2010).

The Proximity Extension Assay

The PEA (Olink Proteomics, Uppsala), is a commercially available platform for high-throughput multiplex biomarker analysis. Combining the high specificity of an immunoassay with the high sensitivity of PCR, PEA is based on pairs of antibodies that are linked to oligonucleotides with a slight affinity to one another (PEA probes). Upon target binding the probes are brought into proximity, and the two oligonucleotides are extended by a DNA polymerase forming a new sequence that acts as a surrogate marker for the specific antigen. The sequence is then quantified by quantitative real-time PCR (qPCR). The number of PCR templates formed is proportional to the concentration of antigen in the sample. The 96-plex PEA allows the detection of 92 protein

biomarkers in 96 samples simultaneously (Assarsson et al. 2014). Olink's tailored 92-biomarker panels target a wide range of disease areas and biological processes. https://www.olink.com.

In recent years, PEA technology has been employed for the identification of novel protein biomarkers and biomarker combinations for the early detection and diagnosis of ovarian cancer (Boylan et al. 2017, Enroth et al. 2018, Skubitz et al. 2019, Enroth et al. 2019). The addition of inflammatory and immunological biomarkers to CA125 and HE4 holds potential to improve the sensitivity and specificity of the established biomarker algorithms.

Epithelial ovarian cancer treatment

The standard care for primary EOC is surgery with platinum-taxane chemotherapy. Centralisation to high-volume tertiary hospitals and surgeons subspecialised in gynaecological oncology have improved the surgical outcome and survival in ovarian cancer (du Bois et al. 2009, Kumpulainen et al. 2009, Bristow et al. 2010, Fagö-Olsen et al. 2011, Dahm-Kahler et al. 2016). Since 2012, the Swedish national guidelines have recommended centralisation of ovarian cancer treatment (RCC 2019).

Surgical treatment

Borderline ovarian tumours and early EOC (FIGO I-IIA)

In patients with ovarian borderline tumours or localised EOC the diagnosis will often not be clear preoperatively. Surgery may be performed as a two-step procedure starting with laparoscopic salpingo-oophorectomy for histopathologic diagnosis. If EOC is confirmed by histopathology, the patient is surgically staged in a second procedure which includes hysterectomy, bilateral salpingo-oophorectomy, omentectomy, systematic pelvic and paraaortic lymphadenectomy, peritoneal biopsies, peritoneal lavage and, in the case of mucinous tumours, appendectomy. In the case of borderline tumours, lymphadenectomy and appendectomy can be omitted (Colombo et al. 2019). In tumours with low-grade endometrioid, LGSC or mucinous histology, systematic lymphadenectomy may be omitted as the risk of lymph node metastases is low (Minig et al. 2017). Staging can be performed by laparotomy or laparoscopy. However, since good-quality evidence to support the benefits of laparoscopic surgery versus laparotomy in early-stage ovarian cancer is lacking, laparotomy is currently recommended (Falcetta et al. 2016, Colombo et al. 2019).

Fertility-sparing surgery

In women of fertile age with borderline tumours or early EOC who wish to preserve their fertility, fertility-sparing surgery can be an option. In invasive cancer, this option is limited to women with stage IA or IC low-grade epithelial tumours (low-grade serous or endometrioid tumours or mucinous histology), and only after complete staging, while fertility-sparing surgery can be considered in selected patients with borderline tumours up to stages II and III (du Bois, Heitz and Harter 2013, Colombo et al. 2019). If fertility-sparing surgery is performed, radical surgery is recommended after child-bearing, to lower the risk of recurrence.

Advanced EOC (FIGO IIB-IV)

The most important prognostic factor in the treatment of advanced EOC is the extent of residual tumour after surgery (Griffiths 1975). It is now widely accepted that patients with no gross residual disease after surgery have superior survival compared to patients with even a small volume of residual disease (< 1 cm) (Chi et al. 2006, Aletti et al. 2006, Chang et al. 2013). Aiming for complete resection of all visible tumours, EOC surgery has evolved from "simple" cytoreductive procedures including total hysterectomy, bilateral salpingo-oophorectomy, infracolic omentectomy, excision of bulky lymph nodes and peritoneal metastases to encompass more "radical" surgery that may include bowel resection, diaphragm stripping, liver resection, splenectomy, distal pancreatectomy, gastric resection, urinary tract resection, and even intrathoracic surgery in select cases (Chang and Bristow 2012). Systematic lymphadenectomy in advanced EOC is not recommended since the recent LION trial found no benefit on survival from pelvic and paraaortic lymphadenectomy in patients with macroscopically completely resected disease and clinically negative lymph nodes (Harter et al. 2019).

The timing of surgery in the most advanced stages (FIGO stages IIIC-IV) should be discussed in a multidisciplinary team setting involving gynaecological tumour surgeons, gynaecological oncologists and radiologists. Primary debulking surgery (PDS) followed by adjuvant chemotherapy is recommended in patients where optimal debulking is considered feasible, while patients with unresectable disease or severe comorbidity may be considered for neoadjuvant chemotherapy followed by interval debulking surgery (NACT-IDS) (Wright et al. 2016, Colombo et al. 2019). The possible benefits of primary surgery must be weighed against the considerable postoperative morbidity and mortality. So far, two randomised trials have found NACT-IDS to be non-inferior to PDS in regard to survival, but the results have been questioned due to a small proportion of PDS patients with complete resection in both the EORTC-GCG and CHORUS trials (Vergote et al. 2010, Kehoe et al. 2015). An ongoing multicentre randomised trial, the TRUST (Trial of Radical Upfront Surgical Therapy in advanced

ovarian cancer), aims to clarify the optimal timing of surgery in advanced EOC. Results are expected in 2024, after five years' follow-up (Reuss et al. 2019).

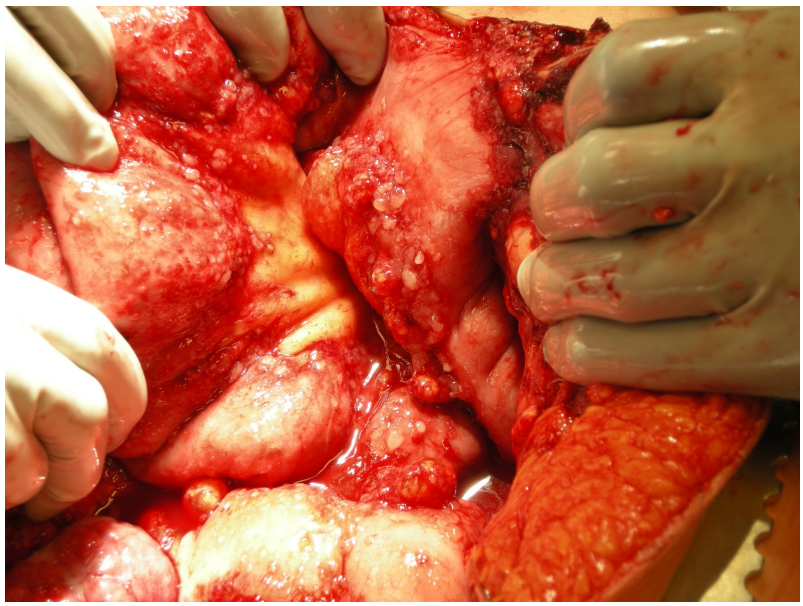

Figure 4. Peritoneal carcinomatosis on the mesentery in advanced EOC. © 2020 Christer Borgfeldt.

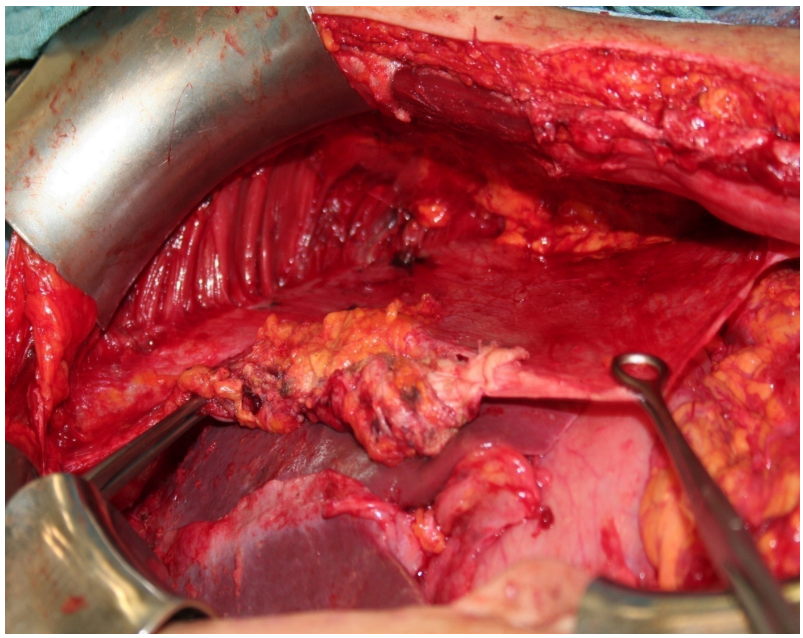

Figure 5. Diaphragm stripping in primary debulking surgery. © 2020 Christer Borgfeldt.

Surgery for recurrent EOC

Chemotherapy is the standard treatment for recurrent EOC, but selected patients with good performance and limited disease may benefit from secondary cytoreductive surgery. Two large randomised trials have recently been performed to assess survival after secondary surgery plus chemotherapy vs. chemotherapy only (in the GOG 213 trial patients were even randomised to chemotherapy + bevacizumab or chemotherapy only). While some positive effect was seen on progression-free survival (PFS) in patients with completely resected disease in the GOG 213 trial, secondary surgery did not result in longer overall survival (OS) (Coleman et al. 2019). In June 2020 the final survival analysis in the DESKTOP III study was disclosed, showing a positive effect of secondary surgery with more than 12 months' gain in OS in patients with complete resection (A du Bois, webcast https://www.youtube.com/watch?v=ZQ5ePwbnk8M). The final results are yet unpublished and therefore should be interpreted with caution. Also, importantly, while these findings support the role of secondary surgery as an option in selected patients with first relapse, the DESKTOP III trial was designed before the introduction of PARP inhibitors in EOC, see below.

Oncological treatment

Chemotherapy

Adjuvant chemotherapy improves survival in EOC and patients with optimal debulking surgery benefit most from chemotherapy. Unfortunately, despite high initial response rates of 80% after first-line treatment, the majority of patients relapse within two years of diagnosis (Agarwal and Kaye 2003). Salvage therapy has improved OS by prolonging the time to the next relapse, and today patients may live through several relapses (Parmar et al. 2003, Pfisterer et al. 2006, Oronsky et al. 2017). A beneficial effect on PFS as well as OS was found for up to the fourth line of treatment in a study by Hanker et al. 2012 (Hanker et al. 2012). However, with more courses of treatment, drug resistance develops, resulting in diminishing response rates and shorter treatment-free intervals and in the end treatment failure and death in more than 90% of patients (Agarwal and Kaye 2003).

Borderline ovarian tumours

There is no evidence for a beneficial effect of adjuvant chemotherapy in borderline tumours, regardless of stage or the presence of peritoneal implants. Chemotherapy is not recommended in the primary treatment of borderline tumours (Fischerova et al. 2012, Colombo et al. 2019).

Primary EOC

Chemotherapy regimens have improved since the 1960s and 70s when postoperative radiotherapy for ovarian cancer was replaced by chemotherapy with a single alkylating agent (in Sweden melphalan) (Smith, Rutledge and Delclos 1975). In Sweden, combination therapy with melphalan and doxorubicin replaced single drug therapy in the late 1970s (Tropé 1987). With the introduction of cisplatin the cisplatin-doxorubicin combination became the standard treatment in the 1980s (Young et al. 1979, Thigpen et al. 1979) (Högberg, Carstensen and Simonsen 1993, Tropé et al. 1996). During the 1990s, carboplatin replaced cisplatin, with lower toxicity (Alberts et al. 1992). The taxanes were introduced in the late 1990s (McGuire et al. 1996, Högberg et al. 2001, Ozols et al. 2003, du Bois et al. 2003). Carboplatin-paklitaxel continues to be the standard therapy for primary EOC in Sweden today.

In early-stage EOC (FIGO I), adjuvant chemotherapy can be omitted in patients with low-risk histology including low-grade endometrioid IA-B, LGSC IA-B and mucinous cancer IA-C. In Sweden, the recommendation for stage I patients with high-risk histology (HGSC, clear cell cancer, undifferentiated cancer, high-grade endometrioid cancer and carcinosarcoma) and complete surgical staging is single therapy with carboplatin 6 cycles. In patients with incomplete staging, carboplatin-paklitaxel combination therapy is recommended. In FIGO II-IV, carboplatin-paklitaxel is recommended for all patients regardless of histology (RCC 2019).

Recurrent EOC

Second- or higher-line treatment is determined by the platinum-free interval. Patients who relapse more than six months after platinum-based chemotherapy are considered platinum-sensitive and many will respond to another line of platinum-based chemotherapy. Patients with relapse during or within six months after platinum-based chemotherapy are considered to have platinum-resistant disease. Chemotherapy without platinum can be offered, in most cases as single therapy with paklitaxel, pegylated liposomal doxorubicin, gemcitabine, etoposide or cyclophosphamide. However, response rates are in general poor and toxicity must be weighed against quality of life (Davis, Tinker and Friedlander 2014, RCC 2019).

Molecular therapy

In the last decade, new categories of drugs have been introduced into the treatment of advanced primary and recurrent EOC.

Angiogenesis inhibitors

The vascular endothelial growth factor (VEGF) is a key regulator of tumour angiogenesis. Angiogenesis inhibitors, including bevacizumab, an anti-VEGF

monoclonal antibody, inhibit tumour growth (Ferrara et al. 2004). In patients with primary advanced EOC (FIGO III-IV) with incompletely resected disease, bevacizumab prolongs PFS, but not OS (Burger et al. 2011, Perren et al. 2011, Tewari et al. 2019). In platinum-sensitive relapse, bevacizumab improves PFS as well as OS, while in platinum-resistant recurrent EOC an effect is only seen on PFS (Pujade-Lauraine et al. 2014, Coleman et al. 2017a).

PARP inhibitors

Around 50% of HGSCs harbour germline or somatic BRCA1 or 2-mutations or other mutations that lead to increased genomic instability due to HRD (Cook and Tinker 2019). HRD increases dependency on DNA single strand break repair, in which poly (ADP-ribose) polymerase (PARP) is a crucial factor and makes the tumour susceptible to PARP inhibition, with increasing DNA damage leading to cell death (O'Connor 2015).

Women with BRCA1 or BRCA2-mutated relapsed HGSC benefit from treatment with the PARP inhibitor olaparib (Ledermann et al. 2014, Kaufman et al. 2015, Ledermann et al. 2016b, Pujade-Lauraine et al. 2017). Recently, the SOLO1 trial has shown olaparib to markedly increase PFS also in primary BRCA1/2-mutated HGSC (Moore et al. 2018). Other PARP inhibitors have entered the market, with trials indicating beneficial effects not only in patients with BRCA mutations. Rucaparib improved PFS in BRCA-mutated as well as non-BRCA HRD recurrent HGSC in the ARIEL3 trial (Coleman et al. 2017b), and in the NOVA trial niraparib improved PFS in recurrent HGSC regardless of BRCA or HRD status (Mirza et al. 2016), widening indications for PARP inhibitors. Since December 2019 niraparib has been offered to Swedish patients with relapsed HGSC and partial or complete response to platinum-combination therapy, regardless of BRCA status. It remains to be seen whether PARP inhibitors will improve OS in EOC.

Aims of the thesis

I. To assess preoperative plasma levels of a panel of four biomarkers (CA125, HE4, B7-H4, suPAR) in relation to EOC types I and II and, secondly, to evaluate the biomarkers' ability to discriminate between benign and malignant tumours and to predict prognosis in EOC.

II. To search for new candidate biomarkers with the potential to improve performance of CA125 and HE4 for discrimination between benign and malignant ovarian tumours.

III. To evaluate ovarian cancer surgery in Swedish tertiary centres and regional hospitals in the period 2013-15.

IV. To analyse time trends for the incidence and survival of epithelial ovarian, fallopian tube, peritoneal, and undesignated abdominal/pelvic cancer in Sweden in the period 1960-2014 with regard to age, tumour site and morphological subgroup.

Material and methods

Paper I

Preoperative peripheral blood samples were obtained from 350 women admitted for primary surgery of an adnexal mass to the Department of Obstetrics and Gynaecology at Lund University Hospital from 1993 to 2010. All diagnoses were verified by histopathological examination. The morphological subtype and FIGO stage of the disease were available in all malignant cases. The survival status of all patients, *i.e.* alive or dead including date of death, was obtained on December 3, 2013 from the Swedish Population Registry.

CA125

Plasma samples were routinely assayed for CA125 using a commercial electrochemoluminescence immunoassay, Elecsys CA125 kit™ (Roche Diagnostics Scandinavia AB, Bromma, Sweden). The assay was performed according to the manufacturer's instructions.

HE4

Plasma HE4 levels were measured with the HE4 assay (Fujirebio Diagnostics, Gothenburg, Sweden). The assay met standard laboratory quality criteria.

Risk of Ovarian Malignancy Algorithm

The ROMA is calculated from menopause status and preoperative levels of HE4 (pmol/L) and CA125 (U/mL) (Moore et al. 2009). For the calculation of the ROMA, please refer to table 3.

uPAR (urokinase plasminogen activator receptor)

The urokinase plasminogen activator (uPA) system is active in inflammation and wound healing, and associated with tissue remodelling, angiogenesis and cell migration. The components of the uPA system are upregulated and have important roles in tumour progression and metastasis in a wide range of cancers including ovarian cancer (Dass et al. 2008). Intact uPAR(I-III) is a three-domain protein, attached to the cell

membrane by a glycolipid anchor to domain III. Binding of uPA to domain I initiates catalysation of plasminogen into plasmin and cleaves uPAR, thereby liberating uPAR(I) and leaving the cleaved and inactive uPAR(II-III) on the surface. Both uPAR(I) and the shed forms of uPAR(I-III) and uPAR(II-III) have been detected in blood (Rasch et al. 2008).

Our group has previously shown that the combination of the plasma soluble uPAR cleavage forms suPAR(II-III) + suPAR(I-III) and CA125 discriminates between malignant and benign ovarian tumours and that suPAR(I) is a prognostic factor in EOC (Henic et al. 2008).

Three uPAR immunoassays, TR-FIA 1, 2 and 3, have been developed by the Gunilla Høyer-Hansens group at the Finsen Laboratory in Copenhagen for the specific measurement of uPAR(I-III), uPAR(I-III) + uPAR(II-III), and uPAR(I), respectively (Piironen et al. 2004). The amount of suPAR(II-III) is obtained by subtracting the moles of suPAR(I-III) measured in TR-FIA 1 from those of suPAR(I-III) and suPAR(II-III) measured in TR-FIA 2.

B7-H4

The B7 protein family has important roles in modulation of T-cell responses. B7-family protein homologue 4 (B7-H4) has an inhibitory effect on T-cell activation. In ovarian cancer, B7-H4 is expressed by tumour-associated macrophages and ovarian tumour cells (Sica et al. 2003, Kryczek et al. 2006). The serum B7-H4 levels are elevated in ovarian cancer but not in other cancers, with higher serum levels in endometrioid and serous than in mucinous EOC. The combination of B7-H4 and CA125 increased performance of detection of early-stage ovarian cancer over the use of CA125 alone (Simon et al. 2006). B7-H4 is a potential prognostic marker in ovarian cancer. A multi-marker panel with KLK7, KLK10, B7-H4 and Spondin-2 was found to predict one-year survival after chemotherapy in the study by Oikonomopoulou (Oikonomopoulou et al. 2008).

The B7-H4 ELISA assay (provided by diaDexus to Fujirebio Diagnostics, Gothenburg, Sweden) met standard laboratory quality criteria.

Statistical methods

Differences between groups regarding plasma levels of the biomarkers were evaluated with the Mann–Whitney *U*-test for unpaired samples, and ANOVA with Bonferroni as *post-hoc* tests, and trends across ordered groups were analysed using linear regression with log-transformed values. Spearman's rho was used as a measure of correlation between the parameters.

Receiver operating characteristic (ROC) curves were constructed, and the area under the curve (ROC-AUC) was calculated with 95% confidence intervals. The diagnostic performance was expressed as sensitivity, specificity and positive (LR+) and negative (LR–) likelihood ratios. For each logistic regression model, a coefficient for each variable included in the model as well as a model constant was determined. Akaike's Information Criterion was used for selecting the best models including interaction coefficients (Akaike 1974). Goodness of fit assessment was carried out using the Hosmer-Lemeshow test. The markers were used as continuous variables in the univariate and multivariate logistic regression models, the binary outcome being benign tumour or ovarian cancer including borderline tumours. Cross-validation of the logistic regression models using a 10-fold-split and the leave-one-out approach was performed to obtain the average ROC-AUC values for combinations of biomarkers. Cross-validation reduces the upward bias in estimating AUC-ROC values in the logistic regression model on the set of patients from which the model was initially fitted. The method described by DeLong *et al. (*DeLong, DeLong and Clarke-Pearson 1988) and bootstraps (n=2000) were used for the calculation of the difference between two ROC–AUCs.

Overall survival probabilities were calculated using the Kaplan-Meier method and the log-rank test. The Cox proportional hazard model was used in univariate and multivariate survival analyses. All comparisons were two-sided, and a 5% level of significance was used.

The statistical analyses were performed using SPSS™ (22.0.0) (Oracle, CA, USA), MedCalc™ (13.1.1.0) (MedCalc Software bvba, Ostend, Belgium) and R (The R Foundation for Statistical Computing, Vienna, Austria).

Paper II

Preoperative peripheral blood samples were obtained from 180 women admitted for primary surgery of an adnexal mass to the Department of Obstetrics and Gynaecology at Lund University Hospital in the period 2005 to 2012. All diagnoses were verified by histopathological examination. Morphological subtype and FIGO stage of the disease were available in all malignant cases. The patient cohort included 30 cases of benign ovarian tumour, 28 cases of borderline tumours, 25 early EOC (FIGO stage I) and 97 advanced EOC (FIGO stages II-IV). The frozen plasma samples were shipped to Olink Proteomics AB, Uppsala, Sweden for PEA analyses.

The Proximity Extension Assay (PEA)

Plasma samples were analysed with the Olink Oncology II and Inflammation panels (Olink Proteomics AB, Uppsala, Sweden), each panel targeting 92 different proteins. As some proteins were included in both panels, a total of 177 unique plasma proteins were analysed. The PEA technology from Olink enables simultaneous analysis of 92 proteins. Pairs of oligonucleotide-labelled antibody probes bind to their targeted protein, and if the two probes are brought into close proximity the oligonucleotides will hybridise in a pairwise manner. The addition of a DNA polymerase leads to a proximity-dependent DNA polymerisation event, generating a unique PCR target sequence. The resulting DNA sequence is subsequently detected and quantified using a microfluidic real-time PCR instrument (Biomark HD, Fluidigm). Data is quality controlled and normalised using an internal extension control and an inter-plate control, to adjust for intra- and inter-run variation. The final assay read-out is presented in Normalised Protein eXpression (NPX) values, an arbitrary unit on a log2-scale where a high value corresponds to a higher protein expression. The NPX values are relative and not comparable between different proteins (Assarsson et al. 2014).

Statistical methods

Hierarchical clustering analysis and principal component analysis were performed to search for clusters of proteins associated with the different tumour categories.

Patients were subsequently dichotomised into benign tumours *vs.* borderline + cancer, or benign tumours + borderline *vs.* cancer, and differences in protein expression between groups were analysed with a Student's t-test with a p-value < 0.001 indicating a statistically significant difference; p-values were adjusted for multiple comparisons using the False Discovery Rate (FDR). Each biomarker was used as a continuous variable in univariate logistic regression models, with the binary outcome benign tumours *vs*. borderline + cancer, or benign tumours + borderline *vs.* cancer. ROC curves were constructed and the AUC was calculated with 95% confidence intervals using the non-parametric bootstrap procedure.

In order to evaluate the biomarkers' potential to improve the established ROMA algorithm (incorporating HE4, CA125 and menopause status) a multivariate logistic regression model including the biomarkers HE4 and CA125 was constructed to serve as a proxy for the ROMA. Each of the remaining biomarkers (AUC > 0.7) was added in turn to the reference model. The classification accuracy of each model was evaluated with the AUC, and the AUC for each model was compared to the AUC of the reference model. A p-value < 0.05 for differences in AUC was considered statistically significant. For each model, the sensitivity was calculated corresponding to a specificity of 0.95. Comparisons were made separately for premenopausal and postmenopausal women

and for the whole patient cohort. As we did not have information on the menopause status of the women we arbitrarily assigned the menopause status as premenopausal: Age < 52 years, and postmenopausal: Age > 51 years at time of surgery, based on the mean menopause age in Sweden of 51.5 years (Rödström et al. 2003).

All statistical analyses were carried out using R v 3.5.2 (R Core Team (2018). The R Foundation for Statistical Computing, Vienna, Austria).

Paper III

The GynOp Registry

The Swedish GynOp Registry started to collect data from patients undergoing gynaecological surgery in 1997. Since 2004, GynOp has included all major gynaecological surgery. The registry is not mandatory and some regions by tradition have reported to another surgical quality registry (Gyn-Kvalitets-Registret, GKR). Gynaecological clinics in four out of six regions in Sweden, covering 5.03 million people or 52% of the Swedish population, reported to the GynOp in 2017.

The GynOp collects data from patient questionnaires and doctors' forms. Patients are identified with their personal number in the register. The patient is included in the registry when surgery is scheduled by the operation planner of the clinic and the patient's reported data are collected in a preoperative questionnaire online or on paper. The doctor reports data to the registry on admission, after surgery and at discharge and when the histopathology results arrive. A postoperative patient questionnaire is sent to the patient eight weeks after surgery. Postoperative complications are reported by the patient in the questionnaire and are subsequently assessed by the surgeon who will grade the reported complication as major or minor according to criteria listed in the GynOp. Surgeons will even report complications to the registry at the time of surgery, discharge or in the event of re-admission or re-operation.

All cases of surgery with curative intent for ovarian, tubal or peritoneal malignancy, including borderline tumour, registered in the GynOp from 2013 to 2015, in total 1,108 cases, were included in the study.

Statistical methods

The Mann–Whitney *U*-test was used for testing differences in distributions between tertiary centres and regional hospitals. Equalities of proportions were tested using a Pearson's chi squared test. Comparisons between tertiary centres were made using ANOVA, a chi squared test or Fisher's exact test where appropriate. Logistic regression

models were constructed to test potential risk factors for complications. All tests were two-sided and a 5% level of significance was used. Statistical analyses were performed using R (v3.2.3, 2015; R core team, Vienna, Austria).

Paper IV

The Swedish Cancer Registry (SCR)

The population-based nationwide SCR started registration in 1958. Coverage is secured by a mandatory requirement for health care providers (both clinicians and pathologists) to register all patients with premalignant and malignant conditions as well as certain benign tumours. The SCR does not include information on treatment. Data are collected by six regional registries. The completeness of registration is over 95%, and 99% of cancer cases are verified by morphology (Barlow et al. 2009). The personal identification number in use in Sweden since 1947 ensures near to complete follow-up of the patients up to the time of death or emigration. The Swedish Cause of Death Registry includes virtually all deaths since 1911, and data can be linked to other national health registries, including the SCR.

A total of 68,819 cases of ovarian, tubal, peritoneal, abdominal, or pelvic malignancy were identified in the SCR 1960 to 2014. After exclusion due to the reasons listed in Fig. 6, 46,350 women aged 18 or older with epithelial ovarian (n=37,538), tubal (n=1,317), peritoneal (n=547) and undesignated abdominal/pelvic cancer (n=6,948) were included in the study. We did not exclude any women on the basis of previous or subsequent diagnoses of other tumours (Coleman et al. 2011).

Follow-up for death was available for all patients up to 30 April 2016. Survival time was calculated from date of diagnosis until date of death, date of emigration, or 30 April 2016.

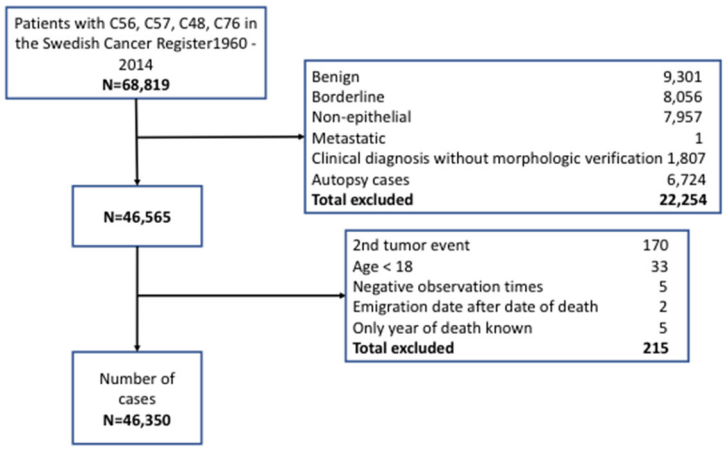

Figure 6. Flow chart for patients who met the inclusion and exclusion criteria of the study. Some cases fulfilled more than one exclusion criterion.

Statistical methods

Incidence rates were standardised to the world standard population 2011 (https://seer.cancer.gov/stdpopulations/). Trends in RS were modelled using flexible parametric models (Lambert and Royston 2009).

For the survival analyses, women were grouped into strata given their age at diagnosis (18–44, 45–54, 55–64, 65–74, and ≥75 years) and period of diagnosis (1960–1964, 1965–1969, … 2010–2014). We estimated RS within age strata along with age-standardised relative survival using the International Cancer Survival Standard population (Corazziari, Quinn and Capocaccia 2004). Expected mortality rates, stratified by age and calendar year, were obtained from the Human Mortality Database (http://www.mortality.org), based on data from Statistics Sweden (http://www.scb.se/en/). To examine temporal trends, time since diagnosis and year of diagnosis were modelled using restricted cubic splines with five and three degrees of freedom, respectively. An explanation of the concept of cubic splines for non-linear associations in clinical practice is given in the paper by Gauthier et al. 2019 (Gauthier, Wu and Gooley 2019). We fitted a separate model within each age group, where the effect of time since diagnosis was time varying, with three degrees of freedom. An illustration of the analytic approach (using publicly available data for colon cancer) is available at http://pauldickman.com/software/stata/prediction-out-of-sample/.

Stata 13 (Statacorp, College Station, TX, USA) was used for the statistical analyses.

Ethical issues

Medical research involving human subjects is necessary to gain a deeper understanding of human diseases and to improve prevention, diagnostics and treatment. The Declaration of Helsinki states that the research must adhere to ethical standards that promote and ensure respect for all human subjects and protect their health and rights. Research may only be conducted if the potential benefits of the study outweigh the risks and burdens to the research subjects. Participation must be voluntary and informed consent must be obtained from the subject, who must also be informed of the right to refuse or withdraw consent at any time (World Medical Association 2013).

To the women who participated in the four studies of this thesis, the outcome of the studies will not have any impact on treatment or prognosis. However, given the lethal nature of ovarian cancer, the potential benefits if diagnostics can be improved for future patients are considered to vastly outweigh the potential harm and discomfort caused by a peripheral blood sample (paper I and II). The medical and surgical registries of Sweden are high-quality sources of population-level information about disease patterns, treatment outcome and more. While registry research involves no risk of physical harm, the risk of harm from dissemination of personal information must be minimised. It is the responsibility of the researcher to protect the privacy and integrity of the research subjects (World Medical Association 2013). To ensure the confidentiality of personal information, in paper III data from the GynOp Registry was anonymised before statistical analyses. In paper IV, data extraction and analysis from the SCR was performed on a group level, with no access to individual patient data.

For the biomarker studies (papers I and II), oral and written informed consent was obtained from the participating women at the time of admission to the department of Obstetrics and Gynaecology, Skåne University Hospital, Lund. For study III, written informed consent to the use of patient data for medical research was obtained from the patients at the time of registration in the GynOp Registry. The patient can request data be withdrawn from the registry at any time. For study IV, no patient consent was required.

Studies I (DNR 558-2004 and DNR 94-2006), II (DNR 495-2016, amendment to DNR 558-2004 and 94-2006) and IV (DNR 789-2015) were approved by the Regional Ethics Committee of Lund University.

Approval for study III was obtained from the Regional Ethics Committee of Umeå University (DNR 2013-155-32M, supplement to 08-120M).

Results

Paper I

The patient cohort included 211 cases of benign ovarian tumour, 30 borderline tumours, 35 EOC type I and 74 EOC type II. The median age was 54.5 years for the whole cohort (range 16-90 years), and 63.1 years in the subgroup of patients with EOC (range 31-87 years). In patients with invasive tumours, the median follow-up time after surgery was 42 months (range=0.2-229 months).

The plasma levels of B7-H4 were higher in patients with EOC II tumours than in those with benign tumours, but no significant differences were found between EOC I or borderline tumours and benign tumours. The suPAR(II-III) levels were significantly lower in benign tumours compared to borderline, EOC I and EOC II tumours. HE4 and CA125 levels were significantly different in all comparisons between benign, borderline, EOC I and EOC II (Fig. 7).

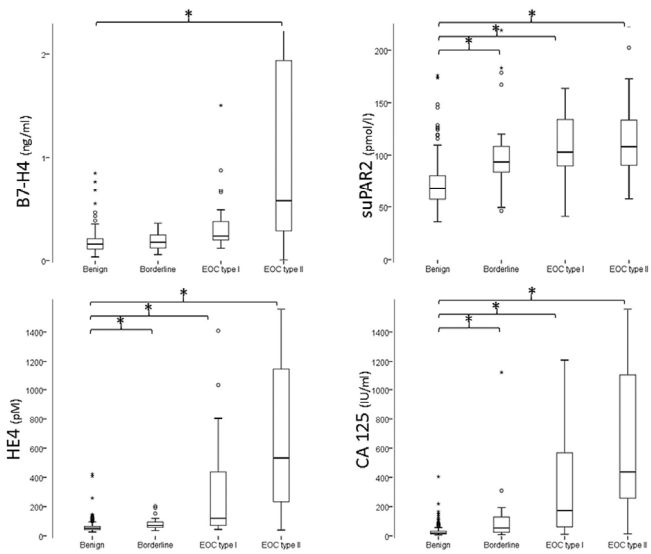

Figure 7. Box plots of preoperative plasma levels of B7-H4, suPAR(II-III), HE4 and CA125 in patients with ovarian tumours. The boxes represent the 25th, 50th, and 75th percentiles. Bars include the highest and lowest values, except outliers (○), which are 1.5- to 3-box lengths from the end of the box, and extremes (*) which are more than three box lengths from the end of the box. All values are included in the analyses but some extreme values are excluded from the figures.
*Significantly different in ANOVA test with Bonferroni post-hoc test ($p<0.05$).

Uni- and multivariate logistic regression models were fitted and performance compared for pre- and postmenopausal women. For women with no information on menopause status, 51.8 years, the median menopause age in Sweden, was chosen as a cut-off. In premenopausal women, the multivariate model including the three biomarkers HE4, CA125 and suPAR(II-III), and age had the best classification accuracy of all models (table 5).

The coefficients in the model including suPAR(II-III), HE4, CA125 and age in premenopausal women were:

$$PI = -36.6 + 3.16 \times \ln(HE4) + 7.96 \times suPAR(II\text{-}III) - 8.75 \times \ln(CA125) + 1.64 \times age + 2.63 \times \ln(HE4) \times \ln(CA125) - 0.37 \times \ln(HE4) \times age$$

Table 5. Comparison of ROC-AUC. Discrimination between benign and malignant including borderline ovarian tumours.

			Pairwise comparison of ROC-AUC*	
			(DeLong)	(bootstraps 2000)
	AUC	95%CI	p-Value	p-Value
Premenopausal				
B7-H4	0.682	0.532-0.832	0.00009	0.00008
suPAR(II-III)	0.822	0.708-0.936	0.0036	0.0028
HE4	0.761	0.620-0.901	0.0014	0.0012
CA125	0.864	0.783-0.946	0.006	0.006
ROMA	0.773	0.633-0.912	0.007	0.005
Model incl lnHE4+lnsuPAR(II-III)+lnCA125+age (10-fold split)	0.940	0.902-0.980		
Model incl lnHE4+lnsuPAR(II-III)+lnCA125+age (leave-one-out)	0.933	0.877-0.989		
Postmenopausal				
B7-H4	0.795	0.724-0.865		
suPAR(II-III)	0.747	0.670-0.825		
HE4	0.880	0.828-0.932		
CA125	0.889	0.833-0.945		
ROMA	0.914	0.867-0.961		
Model incl lnHE4+ lnCA125	0.910	0.861-0.960		
Model incl lnHE4+lnCA125+lnsuPAR(II-III)	0.910	0.861-0.959		
Model incl lnHE4+ lnCA125+lnB7-H4	0.911	0.862-0.959		
Model incl lnHE4+lnsuPAR(II-III)+lnCA125+age	0.912	0.864-0.960		

CI: Confidence interval; ln: natural logarithm. *Model incl lnHE4+lnsuPAR(II-III)+lnCA125+age (leave-one-out) *vs.* other model.

In postmenopausal women, the highest AUC was found for the ROMA. No model with B7-H4 and/or suPAR performed better than the ROMA (table 5).

For the survival analyses, borderline tumours were excluded and the biomarkers were dichotomised to discriminate between high and low risk for OS in univariate Cox regression analysis. uPAR(I) was chosen since in our earlier study, uPAR(I) had shown a higher predictive value for OS than suPAR(II-III) (Henic et al. 2008). At five years from diagnosis, high levels of uPAR(I), HE4 and CA125 were associated with shorter OS. Patients with high levels of HE4 (>400 pM) and/or CA125 (>400 IU/ml) had a median OS of 30 months compared to patients meeting neither of these criteria, where the median OS was 42 months.

The short-term OS was analysed separately at 12 months. suPAR(I) was the only independent preoperative biomarker indicating poor short-term OS. Women above 75 years of age with high suPAR(I) levels had a very poor prognosis (Fig. 8).

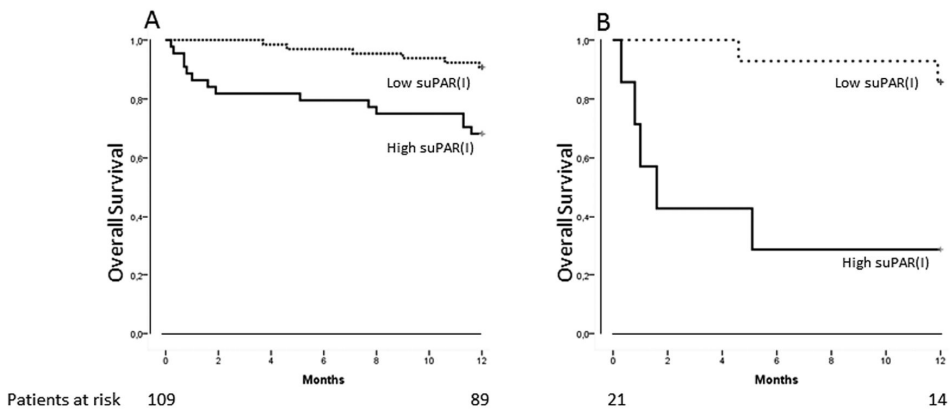

Figure 8. Kaplan-Meier estimates of 12-month overall survival probabilities in patients with suPAR(I) >20.2 pmol/l vs. suPAR(I) ≤20.2 pmol/l. A: Whole patient cohort. B: Patients aged >75 years.

Paper II

Out of the 180 patient samples, eight did not pass internal quality control in the PEA analyses and were excluded from the statistical analyses. The analyses below include 172 patients.

Benign tumours vs. borderline + cancer

Out of the 177 biomarkers analysed, a statistically significant difference in NPX levels between benign tumours and borderline + cancer was found for only two proteins, HE4 and CA125, with higher levels seen in borderline + cancer patients.

Seventeen proteins including HE4 and CA125 had AUC > 0.7 for discriminating benign tumours from borderline tumours + cancer. HE4 ranked highest with AUC 0.771 (95% CI 0.679 - 0.863).

The AUC of a reference model with HE4 and CA125 (AUC 0.773 (95% CI 0.688 – 0.866) all women) was compared with the AUC of the reference model with the addition of each one of the remaining 15 proteins with AUC > 0.7, in a three-biomarker model. Comparisons were made for the whole patient cohort and for premenopausal and postmenopausal women, respectively. The AUC for the reference model was higher for postmenopausal women compared to premenopausal women. Most biomarkers increased the AUC of the reference model but no additional biomarker led to improvement above the significance threshold ($p < 0.05$).

Benign tumours + borderline vs. cancer

Thirty-eight proteins showed significant differences in NPX levels between benign tumours + borderline and cancer. Most were upregulated in cancer patients although a considerable number of proteins, six out of 38 (16%), were downregulated. Again, HE4 and CA125 ranked highest.

Thirty-two proteins had AUC > 0.7 for discriminating benign tumours + borderline from cancer and HE4 ranked highest with AUC 0.837 (95% CI 0.767 - 0.907).

The reference model with HE4 and CA125 (AUC 0.837 (95% CI 0.766 – 0.907) all women) was compared with the AUC of the reference model with the addition of each one of the remaining 15 proteins with AUC > 0.7, in a three-biomarker model. Comparisons were made for the whole patient cohort and for premenopausal and postmenopausal women. The AUC for the reference model was lower for postmenopausal women compared to premenopausal women. Most biomarkers increased the AUC of the reference model but no additional biomarker led to improvement above the significance threshold ($p < 0.05$).

Paper III

Four TCs (Linköping, Lund, Umeå and Uppsala University Hospitals) reported a total of 770 (69.5%) cases and 21 RHs reported 338 (30.5%) cases of surgery with curative intent for ovarian cancer including borderline tumours in the years 2013-15. We decided to include Örebro University Hospital, serving a small region with a population of 292,000, in the RH group.

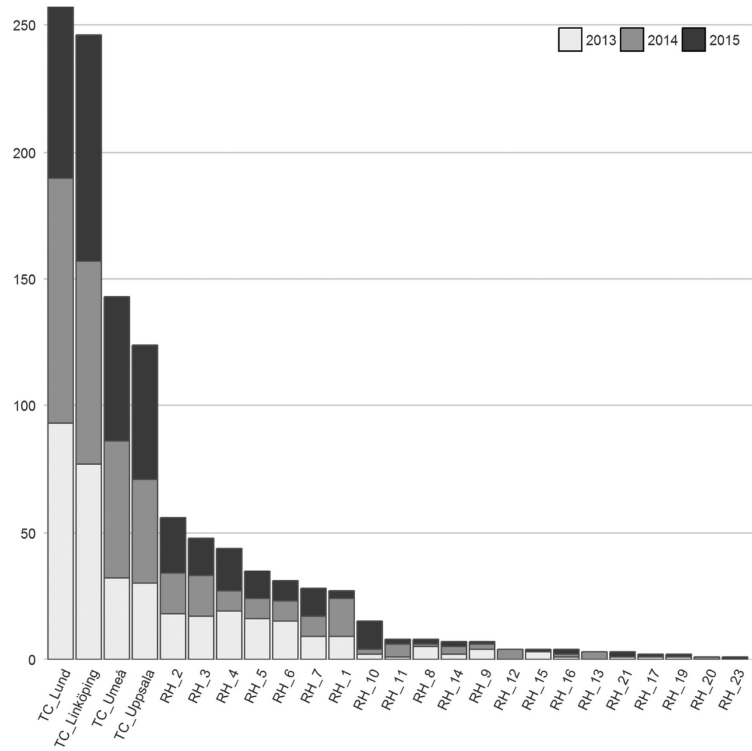

Figure 9. Total number of surgical procedures with curative intent for ovarian/tubal/peritoneal malignancy, including borderline tumours. GynOp 2013 to 2015.

All TCs had a case volume above 25 per year, while no RH reached above 20 cases per year (Fig. 9). More patients were staged with advanced disease, FIGO IIIC-IV, in the TC group, 396 (51.4%) *vs.* 62 patients (18.3%) in RHs. The proportion of patients with RMI > 200, indicating a high risk of EOC, was higher in TCs, 83% compared with 67% in RHs. Information on FIGO stage was available in 88.3% of TC patients and 54.7% of RH patients.

Only patients with advanced disease (FIGO stage IIIC-IV) were included in analyses on surgical outcome.

Out of 458 patients with advanced disease (FIGO stage IIIC-IV), 396 (86.5%) had surgery in TCs and 62 (13.5%) in RHs. Primary Debulking Surgery (PDS) was performed on a higher proportion of patients in RH than in TC (80.9% *vs.* 66.9%. The proportion of patients with no residual tumour after PDS did not differ significantly between TCs and RHs (54% *vs.* 48%). Significant differences were found in the selection of patients for PDS and achievement of no residual tumour after surgery between the four TCs, with PDS performed on 45% to 93% of patients and no residual tumour achieved in 36% to 70% of PDS patients (table 6).

Table 6. Differences between TCs in selection of patients for PDS and IDS. PDS: Primary debulking surgery, IDS: Interval debulking surgery.

Surgery stage IIIC-IV		**PDS**				**IDS**			
	Total	**Total**		**No residual tumour**		**Total**		**No residual tumour**	
	n	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Linköping	136	73	54%	26/73	36%	63	46%	32/63	51%
Lund	134	124	93%	77/124	62%	10	8%	6/10	60%
Uppsala	77	46	60%	32/46	70%	31	40%	21/31	68%
Umeå	49	22	45%	9/22	41%	27	55%	10/27	37%
Total	396	265	70%	144/265	54%	131	33%	69/131	53%

More TC patients had non-gynaecological additional cytoreductive surgery, 44.9% *vs.* 21% of RH patients.

Overall complication rates after surgery were higher in TC; 25.5% *vs.* 13.3% in RH. The most common complication within eight weeks after surgery was infection, affecting twice as many patients in TC as in RH (21.7% *vs.* 10.7%). Major complications were registered in 5.2% of TC patients *vs.* 2.7% of RH patients (n.s.). There were no significant differences among the specific major complications between TC and RH.

A logistic regression model was constructed to evaluate potential risk factors for severe postoperative complications including age, ASA classification, BMI, smoking, care level (TC *vs.* RH), comorbidity, FIGO stage (IIIC-IV yes/no) and surgical treatment (PDS *vs.* IDS). In the whole patient population, FIGO stage IIIC-IV was the only factor associated with increased risk of severe complication, with an odds ratio (OR)=2.38 (1.11-5.45) (p<0.05). Limiting the logistic regression to the FIGO IIIC-IV population revealed no significant risk factors.

Paper IV

The median age at EOC diagnosis in Sweden increased from 59 to 67 years from 1960 to 2014. Stage was first included in the SCR 2004. The majority of ovarian cancer patients were diagnosed in FIGO stages III or IV (53%) while 17% were diagnosed in FIGO stage I and 6.9% in FIGO II. In 24% no information on stage was registered.

From 1980 the overall age-standardised incidence of epithelial ovarian, fallopian tube, peritoneal, and undesignated abdominal/pelvic cancers together decreased markedly in Sweden. The overall decrease in incidence was due exclusively to a declining ovarian cancer incidence, while fallopian tube and peritoneal cancer incidences increased.

Serous carcinomas increased in incidence in the period 1993 to 2014, while endometrioid, mucinous, and undifferentiated carcinomas decreased (Fig. 10).

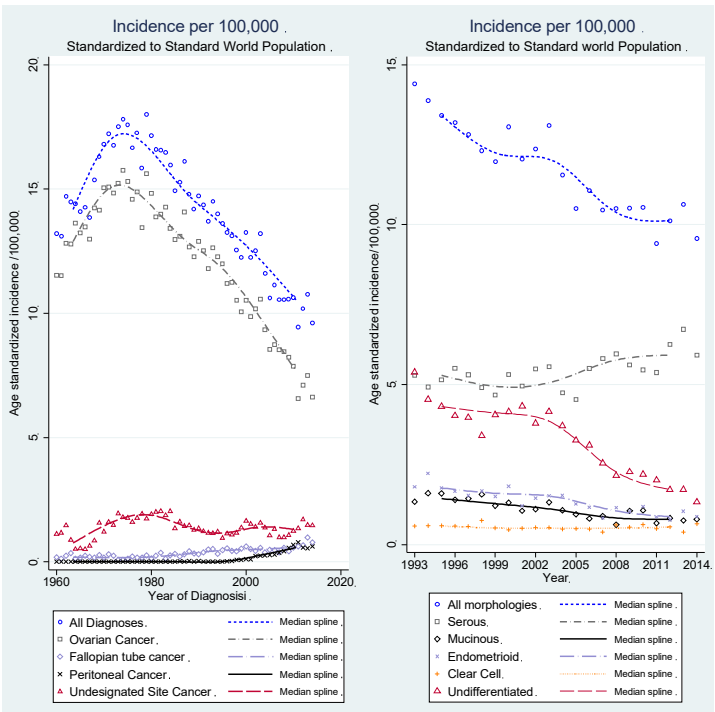

Figure 10. Age-standardised incidence EOC 1960-2014. Left: Tumour site, right: Morphology.

One-, two-, and five-year age-standardised RS improved, especially from the 1980s, while 10-year age-standardised RS did not improve from 1960 (Fig. 11). However, this trend was not seen for the youngest and oldest age groups. For the youngest age group

(18 to 44 years) one- and two-year RS improved only slightly from 1980, converging with survival curves for the older age group (45-54 years). Five-year RS did not improve from 1980, and 10-year RS declined from 1980 to the levels of 1960. The oldest women, 75+ years of age, had the worst RS. One- and two-year RS improved from 1980 onwards but no improvement was found for five-year RS, and 10-year RS declined from 1960.

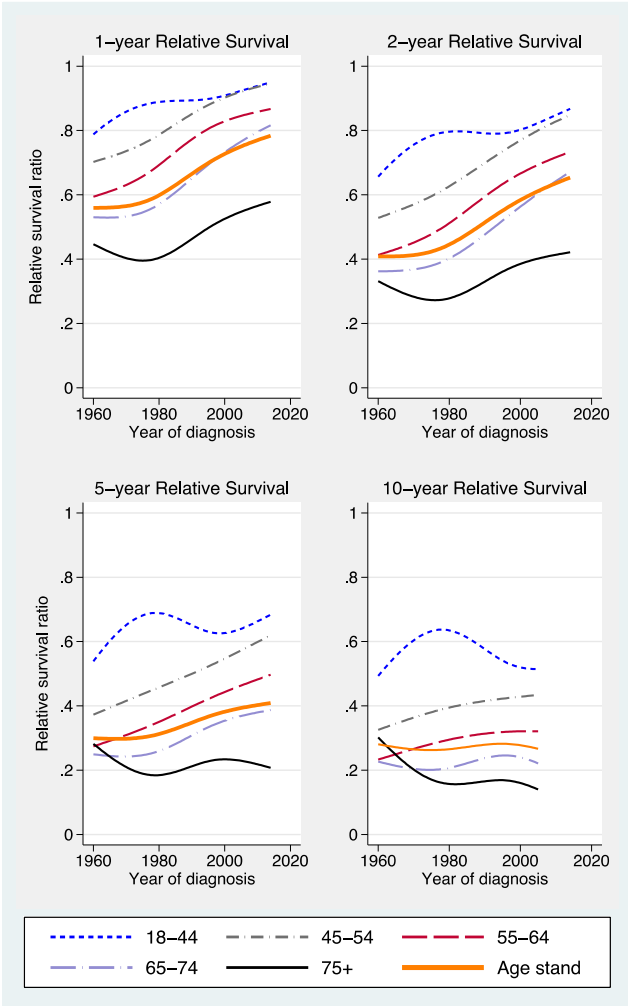

Figure 11. Time trends for one-, two-, five-, and 10-year RS according to age groups and for age-standardised RS. All tumour sites.

Age-standardised RS improved for all sites of tumour origin. However, very few patients were registered with fallopian tube cancer in the early time periods, and none

with peritoneal cancer. The highest RS was seen for fallopian tube cancer, and undesignated abdominal/pelvic cancer had the worst RS.

RS differed between the morphological subtypes, with the highest RS as well as largest improvement in RS found for endometrioid carcinoma. The RS rates increased for all morphologies with the exception of undifferentiated carcinoma, which was also found to have the worst RS.

Discussion

Protein biomarkers for the risk assessment of ovarian tumours

The biomarker algorithms currently in clinical use for the preoperative assessment of an ovarian tumour, the RMI and the ROMA, have low sensitivity and specificity in premenopausal patients and in early-stage and type I EOC (Lennox et al. 2015, Kaijser et al. 2014). Different EOC subtypes express different biomarker patterns, and non-EOC and metastatic ovarian tumours, although rare, contribute to decreasing the diagnostic performance (Köbel et al. 2008, Moore et al. 2009). While epithelial ovarian cancer is a rare diagnosis in premenopausal women, with only 15% of women diagnosed with ovarian cancer below 50 years of age (Socialstyrelsen 2018), early diagnosis is especially desirable in this patient group, not only because it is associated with a markedly improved prognosis but also since early detection will increase opportunities for fertility-sparing surgery, a highly important issue for many women of fertile age. On the other hand, over-diagnosis of suspect ovarian malignancy can result in unnecessary surgical interventions on benign ovarian tumours, putting fertility at risk (Kaijser et al. 2014). Studies I and II in this thesis aimed to search for new biomarkers with potential to improve diagnostics in women with adnexal mass.

In our first study (paper I) we found the plasma levels of suPAR(II-III) as well as HE4 and CA125 to increase from benign tumours to borderline tumours, EOC type I and EOC type II, in accordance with earlier described differences in tumour biology and biomarker expression. The lower levels of HE4 and CA125 in EOC type I and borderline tumours reduce the diagnostic performance of the ROMA in these tumours.

The three-biomarker model with HE4, CA125, suPAR(II-III) and age discriminated between malignant and benign adnexal lesions in premenopausal women, with a diagnostic performance comparable to the IOTA ultrasound-based predictive models (LR2 and the Simple Rules) (Kaijser et al. 2014). The proposed model suggested that adding an inflammatory biomarker known to be involved in carcinogenesis to the established ROMA model has potential to increase the diagnostic accuracy without the addition of ultrasound assessment.

In the second study of this thesis we further investigated the potential of inflammatory biomarkers as well as other known cancer-associated biomarkers to improve the diagnostic performance of CA125 and HE4 in the risk assessment of an adnexal mass (paper II).

Several biomarkers associated with both oncogenic (ITGAV, DNER, CXCL6, ABL1, SCF) as well as tumour-suppressive actions (DNER, CXCL11) were found to increase the diagnostic performance of CA125 and HE4 in our study, but no biomarker improved the diagnostic performance of CA125 and HE4 above the significance threshold ($p<0.05$). While the other mentioned markers have previously been described in EOC, we did not find any previous reports on DNER and CXCL6 in EOC. Earlier studies have described important functions in carcinogenesis of other malignancies, and it is likely that DNER and CXCL6 also play an important role in EOC pathogenesis. Further studies on DNER and CXCL6 in EOC would be of interest, as these tumour markers may also represent possible targets for immunomodulating therapy (Verbeke et al. 2011, Nowell and Radtke 2017).

The AUC for our reference model of CA125 and HE4 was higher for the discrimination of benign tumours + borderline vs. cancer compared to benign tumours vs. borderline + cancer. Our findings illustrate the diagnostic problem of discriminating benign from borderline tumours using protein biomarkers, as biomarker levels may be normal or only slightly elevated in borderline tumours (Moore et al. 2009, Braicu et al. 2014, Kotowicz et al. 2015) and benign ovarian tumours as well as a range of other benign conditions can present with elevated levels of biomarkers (Jacobs and Bast 1989, Moore et al. 2012). Whether this is a matter of concern in the preoperative assessment can be debated, as borderline tumours have an excellent prognosis and will rarely progress to invasive cancer even after conservative surgery for a supposedly benign adnexal mass (Morice et al. 2003). However, in women of fertile age, early diagnosis of a borderline tumour will increase the likelihood of being able to offer fertility-sparing surgery.

Many other research groups have investigated new protein biomarkers and biomarker combinations to improve ovarian cancer diagnostics. The suggested biomarker combinations vary greatly, as do study design and patient populations (Boylan et al. 2017, Han et al. 2018, Enroth et al. 2018, Enroth et al. 2019, Moore et al. 2019). While some groups have reported promising results, the findings of our group and others suggest that new biomarkers add little to the diagnostic performance of CA125 and HE4 in women with ovarian tumours. Ultrasound-based models for the assessment of ovarian tumours remain superior in regard to sensitivity, albeit at the cost of lower specificity (Meys et al. 2016). An alternative and attractive approach, recently suggested by Lycke et al., could be to simply combine the established biomarkers and algorithms.

The combination of the RMI with HE4 and the ROMA with TVS into new algorithms was found to improve specificity at maintained or improved sensitivity (Lycke et al. 2020). However, limited access to ultrasound at primary care level restricts the utility of ultrasound-based models in many countries. In a setting without access to ultrasound assessment, the ROMA continues to appear the most useful model for triage of a patient with an ovarian tumour to the correct care level for further preoperative assessment, including ultrasound. However, in a primary care setting with access to good quality ultrasound, combining biomarker testing (CA125 and HE4) with TVS seems to offer the best initial assessment of the woman with suspected ovarian cancer today.

Prognostic biomarkers in EOC

Both CA125 and HE4 have been assessed for their prognostic potential in EOC. HE4 at diagnosis was found to be a prognostic factor for survival in several studies, including an earlier publication from our group (Kalapotharakos et al. 2012). The ROMA was also predictive for poor survival in a study by Bandiera et al. 2011, while the prognostic value of CA125 at diagnosis is less clear (Zorn et al. 2009, Bandiera et al. 2011, Trudel et al. 2012, Furrer et al. 2019).

In our first study (paper I), we found high levels of preoperative suPAR(I), CA125 and HE4 correlated with poor OS. In multivariate analyses, high suPAR(I) was an independent prognostic biomarker for poor survival at one year from diagnosis. Our results support suPAR(I) as a prognostic marker in EOC, as reported earlier by our group (Henic et al. 2008). SuPAR(I) is an inflammatory biomarker, reflecting the amount of uPA activity, as uPA is needed to liberate suPAR(I). While intact suPAR has previously been investigated in EOC with varying results, we found no other reports on the prognostic value of cleaved suPAR forms (Sier et al. 1998, Riisbro et al. 2001, Begum et al. 2004).

High suPAR(I) levels indicate very poor prognosis in elderly women above 75 years of age in the first year from diagnosis (paper I). More than one out of five ovarian cancer patients (23%) are diagnosed at the age of 75 or above (Socialstyrelsen 2018). High age is correlated with more advanced stage and high-grade disease at diagnosis, as well as higher comorbidity and lower performance status, and older patients more often receive suboptimal treatment. Advanced age is associated with a worse prognosis. However, studies show conflicting evidence regarding age as an independent prognostic factor. Elderly patients who receive optimal treatment have a better prognosis (Gibson et al. 2016, Joueidi et al. 2020). The challenge lies in identifying which patients are likely to tolerate the standard treatment of primary debulking surgery (PDS) and adjuvant

chemotherapy. A geriatric vulnerability score has been evaluated by the French GINECO group for the identification of vulnerable patients among elderly women with advanced EOC scheduled for first-line chemotherapy, with promising results (Falandry et al. 2013, Tinquaut et al. 2016). A vulnerability score for use in the preoperative assessment of elderly women would be of great value for guiding treatment decisions in this frail patient group. Noer et al. have developed an ovarian cancer comorbidity index for use in the preoperative assessment of ovarian cancer patients (Noer et al. 2016). Especially in older women, the addition of biomarkers such as HE4, CA125 and eventually uPAR(I) to the preoperative assessment may aid in deciding between extensive primary surgery or neoadjuvant treatment.

Centralisation of ovarian cancer surgery

The single most important prognostic factor in ovarian cancer surgery is the amount of residual tumour after surgery (Chang et al. 2013). In order to achieve the goal of complete resection, many patients with advanced EOC need extensive abdominal surgery (Chang and Bristow 2012), and several studies have confirmed that centralisation of ovarian cancer surgery to high-volume, gynaecological oncology centres improves surgical outcome and survival (Kumpulainen et al. 2009, du Bois et al. 2009, Bristow et al. 2010, Fagö-Olsen et al. 2011, Dahm-Kahler et al. 2016).

Our study (paper III) shows that following the decision to centralise ovarian cancer surgery in Sweden in 2012, a considerable proportion of ovarian cancer surgery continued to be performed outside TCs. In the regions reporting to the GynOp Registry, 30% of patients with ovarian malignancy had their surgery at a RH with less than 20 cases per year in 2013 to 2015. TCs performed more extensive surgery without an increased frequency of major complications compared with RHs.

Four TCs performed more than 25 procedures per year, with unexpectedly large differences in patient selection for PDS in advanced EOC (45% to 93%) and complete resection rates in PDS (36% to 70%). PDS is recommended as the standard of care in advanced EOC, while neoadjuvant chemotherapy should be considered only for patients with non-resectable tumours or high comorbidity (Wright et al. 2016, Colombo et al. 2019). Given the public health care system in Sweden it is unlikely that differences in patient referral patterns would explain these differences. We believe that the differences in patient selection for PDS, and even in achieving complete resection, must therefore be due to different surgical cultures at the different TCs.

While we did not have the opportunity to assess survival outcomes in our study, another Swedish study by Dahm-Kahler et al. 2016 reported a markedly improved surgical outcome with an increased proportion of PDS and complete resection as well as improved survival after centralisation in Western Sweden (Dahm-Kahler et al. 2016). Our data indicate an urgent need for further centralisation and, perhaps most importantly, increased collaboration and exchange of knowledge between TCs, to ensure Swedish women with ovarian cancer have equal access to high-quality care, regardless of their region of living. The Swedish three-year subspecialist programme for gynaecological oncology surgery, implemented since 1999, is one important measure for improving quality in ovarian cancer surgery (du Bois et al. 2009). Another could be to further reduce the number of the centres for ovarian cancer surgery in Sweden, currently seven, for increased surgical volume per centre and surgeon (Kumpulainen et al. 2009, Bristow et al. 2014). However, in a country the size of Sweden the benefits of high-volume centres must be weighed against the longer distance to hospital for many patients, which may cause some, especially elderly patients, to abstain from surgery.

EOC incidence and survival in Sweden

Incidence

EOC incidence has declined since 1980 in Sweden (paper IV). Declining incidence is also found in other Northern European countries and North America (Coleman et al. 2011, Coburn et al. 2017). Possible explanations for this decline were discussed earlier in this thesis, with the introduction of the combined oral contraceptives in the 1960s regarded to be a major contributing factor, together with a decline in HRT use and changed pathology classification criteria leading to a diagnostic shift from low-grade carcinoma to borderline tumours (Rossouw et al. 2002, Beral et al. 2008, Skírnisdóttir et al. 2008, Reid, Permuth and Sellers 2017). In the present study we excluded borderline tumours.

Survival

RS up to five years after diagnosis improved during the study period, in parallel with improved surgical and oncological treatment for EOC. Ten-year age-standardised RS, however, did not improve from 1960. Timmermans et al. had similar findings in their study from the Netherlands Cancer Registry of increased five-year survival but essentially unchanged 10-year survival in EOC over 25 years, despite advances in cancer treatment (Timmermans et al. 2018).

There are some notable age exceptions to the overall trend of improved RS. For the youngest age group (18 to 44 years) the lack of improvement coincides with the steep increase in borderline tumour incidence since the 1980s described earlier. Since borderline tumours are more frequently diagnosed in younger women, a shift in diagnostic criteria from low-grade cancer to borderline tumour is likely to exclude a considerably higher number of patients with good prognosis from this age group compared to older age groups (Skírnisdóttir et al. 2008). The poorest RS was found for the oldest women, 75 + years, in line with the findings of other EOC survival studies (Akhtar-Danesh, Elit and Lytwyn 2011, Baldwin et al. 2012, Wright et al. 2015, Lee et al. 2018). Improved one- and two-year RS in this age group may partly depend on much better tolerability to carboplatin-paclitaxel compared to earlier cisplatinum-based regimens in the elderly. In addition, improved peri- and post-operative care has allowed more elderly patients with high comorbidity to be considered for primary surgery. The lack of improvement in five-year RS and the decline in 10-year RS in the elderly may partly be an effect of selection bias. In the period 1960–1964, 8.5% of cases in the SCR were not morphologically verified, compared to only 0.2% of cases in the period 2010–2014. By excluding from the study cohort cases with clinical diagnosis only, it is likely that a larger proportion of patients with more advanced age and disease, and poorer prognosis, were excluded from analyses in the earlier time periods.

Serous carcinoma was the most prevalent morphologic subtype in the current study. Unfortunately, as registration of grade of differentiation was first included in the SCR of 2016, in our analyses we did not have the opportunity to differentiate between LGSC and HGSC, which are today regarded as two subtypes with different origins and clinical features. LGSC constitutes less than 5% and HGSC around 70% of EOC, according to Prat et al. (Prat 2012).

While LGSC in most cases has a good prognosis, HGSC is responsible for 90% of EOC deaths (Kurman and Shih 2011). However, HGSC has proved to be the subtype most responsive to chemotherapy, especially the platinum-taxane combination treatment (McCluggage 2011). Also, as HGSC in most cases presents with disseminated disease at diagnosis, patients with serous histology are most likely to benefit from the shift towards more extensive cytoreductive ovarian cancer surgery that occurred from 2005 onward. Around 50% of HGSC harbours BRCA1/2-mutations or other mutations that lead to homologous recombination deficiency (HRD) and dependency on poly (ADP-ribose) polymerases (PARP) enzymes for DNA repair. The introduction of PARP inhibitors during the last decade is expected to improve survival considerably in these patients (Cook and Tinker 2019). However, as PARP inhibitors were first included in standard care recommendations in Sweden in 2015, this will not have affected survival trends in the current study, with follow-up ending in 2016.

In summary, we show in paper IV that the incidence of EOC has declined since 1980 in Sweden. The age-standardised one-, two-, and five-year RS improved from 1960 to 2014, although this trend was not found in the youngest and the oldest women. The 10-year RS did not improve in women diagnosed from 1960 to 2005. The observed improved short-term RS since 1960 can be explained by improved surgical techniques, better postoperative and advanced intensive care, and more efficient and tolerable chemotherapeutical regimes together with improved supportive care during chemotherapy. Advances in treatment have prolonged life after diagnosis but long-term survival in EOC remains poor. It is hoped that new targeted treatments, including PARP inhibitors, will improve long-term survival in these patients. However, a cure for advanced ovarian cancer seems still far away. Efficient strategies for screening and early diagnosis are urgently needed in order to improve ovarian cancer survival.

Methodological considerations

Paper I

Our patient cohort comprised women consecutively admitted to surgery for ovarian tumours at Lund University Hospital. As Lund is a tertiary centre for gynaecological oncology surgery, selection bias leads to the proportion of women with ovarian malignancy being considerably larger than what would be found among women presenting with an ovarian tumour in the primary care setting. The stage distribution differed between EOCs I and II, with a higher proportion of early-stage in EOC I patients, reflecting the indolent behaviour of EOC I (Kurman and Shih 2016). The stage distribution is expected to contribute to the lower biomarker plasma levels in EOC I vs EOC II, as the levels of all biomarkers were strongly correlated to stage. Age is another likely confounder, as median age differed for the whole patient population versus women with EOC. We did not adjust for stage or age as confounding factors in the analyses of biomarker trends or ROC-AUC. The menopausal status was unknown in some patients. To adjust for this, we tested different menopausal cut-off ages in repeated analyses. Changing the cut-off for age did not significantly change the ROC-AUC values and the median menopausal age in Sweden, 51.8 years, was chosen for the analyses. This arbitrary cut-off is bound to cause misclassification bias. However, while this may be considered by some to rule out any comparison with the ROMA, a modified version of the ROMA, incorporating age instead of menopause status, has been validated in the CPH-Index. The CPH-I was shown to perform comparably to the ROMA and the RMI in a tertiary setting (Karlsen et al. 2015).

Due to the small sample size, we were not able to split the cohort into training and validation cohorts. To reduce the risk of over-fitting our model by using the same cohort for development and validation of the model, the 10-fold split and leave-one-out methods were used for cross-validation. Still, the lack of a validation cohort, together with a highly selected patient population, limits the external validity of our model.

The long follow-up time and consistency in treatment regimens increased the reliability of our survival analyses. The Swedish Population Register ensured complete follow-up of all the patients. OS was chosen as the only end-point, since PFS is dependent on variables such as follow-up intervals and other parameters chosen to indicate progression. Death among patients diagnosed with ovarian cancer is to a large extent related to progression of the malignant disease.

Paper II

In study II our study population was designed to include equal proportions of benign, borderline and early-stage EOCs (FIGO stage I). The patients with borderline tumours and early-stage EOC were few, resulting in 25-30 patients in each of the three categories while the proportion of late-stage EOC equalled the other three categories combined. While this design strengthens the comparison of biomarker performance between subgroups, the results will not be generalisable to the clinical setting. Our research was intended as a pilot study, with a larger follow-up study needed for validation in the event of positive findings. However, no new biomarkers were identified. Low sensitivities and wide confidence intervals indicate high statistical uncertainty in our results, causing low internal as well as external validity. It is possible that a larger study size would have caused some of our findings to reach above significance threshold. We did not have access to data on menopause status, a requirement for the calculation of the ROMA. Instead, we arbitrarily assigned menopause status according to the median menopause age in Sweden, to a fixed cut-off of <52/> 51 years. Arguments for and against this approach are given above (paper I).

The strengths of study II include the use of a validated commercial assay, with high sensitivity and specificity for biomarker analyses. The samples were analysed at the Olink lab facilities, ensuring a high standard of analysis. Out of 180 patient samples, only eight did not pass internal quality control.

Paper III

Since not all regions in Sweden report to the GynOp Registry, we had no information on the situation of ovarian cancer surgery in the remaining regions of the country, including the major city regions of Stockholm and Gothenburg. Due to this, our

findings may not be generalisable to the state of ovarian cancer surgery in these regions. Furthermore, the pattern of referrals from the RHs to TCs could not be analysed since this information is not included in the registry. However, given the public health care system of Sweden, the vast majority of patients will be referred to the tertiary centre of their residential region for surgery, ensuring a comparable referral pattern to the different tertiary centres. There was a considerable amount of missing data in the registry, affecting the internal as well as the external validity of our findings. Due to short follow-up, PFS or OS could not be evaluated.

The use of prospectively collected data reduces the risk of selection bias. The patients' reported response rate was high (85%) in the postoperative questionnaires. The use of questionnaires has been shown to provide more complete and thorough postoperative information than follow-up visits. (Ladfors et al. 2002). However, some recall bias is expected with the use of questionnaires.

Paper IV

By excluding from the study cohort cases with clinical diagnosis only, it is likely that we have excluded a considerable proportion of patients with more advanced age and disease, and poorer prognosis from analyses in the early time periods. As discussed above, this selection bias may have impacted the results of our survival analyses. Other limitations of our study are the lack of a central pathology review, and lack of specification of the grade of differentiation. Improved histopathological classification criteria during the study period results in considerable information bias and must be taken into consideration in the interpretation of the observed incidences and survival rates. As the SCR does not contain information on treatment, we could not directly correlate survival trends to the prevailing surgical and oncological treatment strategies of the different time periods.

The strength of our study is our nationwide SCR cohort, with over six decades of longitudinal data. The use of age-standardised incidences and age-standardised relative survival in analyses minimises confounding from changing population patterns and mortality from other reasons over time, increasing the internal and external validity of our findings. Also, the inclusion of undesignated abdominal/pelvic cancer in the survival analyses provides a more valid estimate of EOC survival over time compared to earlier survival studies. To our knowledge, the study by Dahm-Kähler et al. 2017 is the only previous one to have included these patients in survival analyses (Dahm-Kahler et al. 2017). Lastly, the use of flexible parametric models for survival analysis offers a more dynamic estimate of survival compared to traditional survival analysis using Cox regression and Kaplan-Meier curves.

Conclusions

- The plasma levels of CA125, HE4 and suPAR(II-III) increase from benign tumours to borderline tumours, EOC type I and EOC type II. B7-H4 is increased in EOC II compared with benign ovarian tumours (paper I).
- A biomarker panel with suPAR(II-III), HE4, CA125 and age discriminates benign from malignant ovarian tumours including borderline tumours with higher diagnostic accuracy compared to the ROMA in premenopausal women. The ROMA performs better in postmenopausal women (paper I).
- High suPAR(I) predicts poor short-term survival after EOC diagnosis, especially in elderly women (paper I).
- Out of 177 biomarkers, HE4 was the best performing marker for discrimination between benign and malignant ovarian tumours. No biomarker significantly improved the diagnostic performance of HE4 plus CA125 (paper II).
- In 2013-15, 30% of patients with ovarian malignancy had primary surgery at regional hospitals that saw fewer than 20 cases per year. Four tertiary centres performed more than 25 surgeries per year in the regions reporting to the GynOp Registry (paper III).
- Compared with regional hospitals, tertiary centres perform more extensive surgery without an increased frequency of major complications. Significant differences exist in patient selection for PDS and complete resection rates between the tertiary centres (paper III).
- Since 1980 the incidence of EOC has declined in Sweden (paper IV).
- Better surgical and oncological treatment has improved survival in EOC up to five years from diagnosis since 1960. However, long-term survival remains poor (paper IV).

Future perspectives

Many of the protein biomarkers found to be associated with EOC in paper II are or have been under investigation as potential targets for molecular or gene therapy, and/or as predictive markers for response to treatment. Examples of biomarkers targeted in clinical ovarian cancer trials include ABL1 (the tyrosine kinase inhibitor imatinib mesylate)(Coleman et al. 2006, Noguera et al. 2012), VEGFR-2 (the tyrosine kinase inhibitor cediranib)(Ledermann et al. 2016a) and CD40 (the CD40 antibody selicrelumab)(Piechutta and Berghoff 2019). In the near future of personalised medicine, it is likely that we will see protein biomarker screening implemented during the decision-making on the treatment strategy for the individual patient with ovarian cancer.

New categories of biomarkers have emerged in recent years. The future will show whether these new markers can improve ovarian cancer diagnostics and, hopefully, survival.

MiRNAs are short-sequence RNAs that do not encode a protein and that are involved in the post-transcriptional regulation of the expression of the majority of human genes. In ovarian cancer the expression of some miRNAs is inhibited, suggesting they function as tumour suppressor genes, while other miRNAs are overexpressed and can be regarded as proto-oncogenes (Chen et al. 2019). Circulating miRNAs are highly tissue-specific and can identify the origin of metastasis. A large number of miRNAs have been profiled in serum or plasma, and several miRNAs have been investigated as potential diagnostic and prognostic markers or therapeutic targets in ovarian cancer (Langhe 2015). A recent meta-analysis by Cui et al. supports the use of three miRNAs as diagnostic biomarkers in ovarian cancer (Cui, Hong and Zhu 2020).

Circulating cell-free DNA (cfDNA), derived from necrotic or apoptotic tumours as well as non-tumour cells, can be analysed for tumour-specific hyper- or hypo-methylation. The levels of circulating cfDNA are abnormally high in early as well as advanced stage tumours. Several tumour-specific methylation-based biomarkers /biomarker panels with high sensitivity and specificity for early-stage disease have been identified in serum or plasma from ovarian cancer patients, suggesting cfDNA is a promising target for early detection (Singh, Gupta and Sachan 2019).

Another approach is to identify tumour-specific mutations in the fraction of cfDNA derived from tumour cells (circulating tumour DNA (ctDNA)), a strategy currently applied as an alternative to tissue biopsies for tumour genotyping and for guiding treatment decisions in late-stage disease. As yet, limitations exist for the use of ctDNA as an early cancer detection tool, including very low fractions of ctDNA in total cfDNA (<1%) during the early stages of many cancers, often below the detection limit of standard sequencing platforms, and the high costs for multiple sequencing (Campos-Carrillo et al. 2019).

Combining different biomarker categories seems an attractive strategy to optimise sensitivity and specificity. Cohen et al. combined assays for genetic alterations in ctDNA and eight protein biomarkers in their early detection blood test (Cancer SEEK), reaching impressive sensitivities (from 69% (oesophageal cancer) up to 98% (ovarian cancer)) and specificity > 99% for detection of cancer in the ovary, liver, stomach, pancreas, and oesophagus (Cohen et al. 2018).

An ongoing study in our research group investigates ctDNA mutations in plasma and tissue samples from a subgroup of the patients included in study II of this thesis. Mutation patterns will be correlated to protein biomarker expression patterns.

Since the writing of paper III, a decision has been made to dismantle the string for gynaecological surgery for malignant diagnoses in the GynOp Registry. Since January 1, 2020 surgeons report directly to the Swedish Quality Registry for Gynaecological Cancer (SQRGC), while previously, data from the GynOp and the GKR were manually transferred to the SQRGC. It is hoped that the use of a common registry for all gynaecological clinics in Sweden will improve the completeness of data registration and increase the quality of research on registry data. The GynOp continues to be the Swedish quality registry for benign gynaecological surgery, and has recently obtained nationwide coverage.

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